S. Britain

VOL. XXIX, PART III

The British Mycological Society

(Recognosce notum, ignotum inspice)

TRANSACTIONS

Edited by

B. BARNES and W. C. MOORE

Price to non-members, Seven Shillings and Sixpence net

CAMBRIDGE UNIVERSITY PRESS
CAMBRIDGE, AND BENTLEY HOUSE, LONDON
AGENTS FOR U.S.A.
THE UNIVERSITY OF CHICAGO PRESS
CANADA AND INDIA: MACMILLAN

All rights reserved

PRINTED IN GREAT BRITAIN

OFFICERS AND COUNCIL FOR 1946

President

J. Ramsbottom, O.B.E., Dr.Sc., M.A., F.L.S.

Vice-Presidents

G. Smith, M.Sc.
R. W. Marsh, M.A.
C. T. Ingold, D.Sc.

Secretary

G. C. Ainsworth, B.Sc., Ph.D., F.L.S.
Belmont Laboratories, Ventnor Rd., Sutton, Surrey

Treasurer

A. A. Pearson, F.L.S.
Nutcombe House, Hindhead Rd., Hindhead, Surrey

Editors

B. Barnes, D.Sc., Ph.D., F.L.S.
Dept. of Biology, Chelsea Polytechnic, London, S.W.3

W. C. Moore, M.A.
Plant Pathology Laboratory, Milton Rd., Harpenden, Herts.

Council

The Officers, with the following in addition:

Miss M. A. Brett	A. H. Campbell	H. E. Croxall
R. W. G. Dennis	C. G. Dobbs	Miss L. E. Hawker
S. J. Hughes	G. Samuel	F. A. Sowter

Sir R. H. Biffin, Prof. F. T. Brooks, Prof. W. Brown, A. D. Cotton, F. G. Gould, Dame Helen Gwynne-Vaughan, Miss G. Lister, T. Petch, Dr G. H. Pethybridge, Prof. M. C. Potter, E. W. Swanton, Miss E. M. Wakefield.

PLANT PATHOLOGY COMMITTEE

Chairman: W. C. Moore

Secretary: H. E. Croxall, Long Ashton Research Station, Field Laboratory, Abbey Rd., Evesham.

F. C. Bawden	A. Beaumont	K. St G. Cartwright
R. W. G. Dennis	D. L. G. Davies	C. J. Hickman
J. Grainger	N. C. Preston	I. W. Prentice
E. R. Wallace		

The President and the Secretary *ex officio*

The subscription price is £1. 10*s*. per volume (post-free), payable in advance; single numbers 7*s*. 6*d*. net (double numbers 15*s*. net). Subscriptions may be sent to any Bookseller, or to the Cambridge University Press, Bentley House, 200 Euston Road, N.W.1.

The Cambridge University Press has appointed the University of Chicago Press agents for the sale of *The British Mycological Society's Transactions* in the United States of America.

OOGENESIS AND FERTILIZATION IN *ISOACHLYA ANISOSPORA* VAR. *INDICA*

By K. S. BHARGAVA

(With 2 Text-figures)

Introduction

Sexual reproduction in the Saprolegniaceae has been of interest for many years and for long it was a subject of controversy. Trow (1895) was the first to show that fertilization occurred in this family: subsequently, the occurrence of fertilization has been demonstrated in other genera of the family. For a review of the literature the reader is referred to Shanor (1937) and Wolf (1938).

The purpose of this study has been to follow the development of the sexual organs in living condition and to make a cytological study of fertilization in *Isoachlya anisospora* (de Bary) Coker var. *indica* Sak. & Bhar., a genus hitherto uninvestigated.

Material and methods

The material was obtained from a local pond (Saksena & Bhargava, 1944). Cultures of the fungus were maintained on halves of hempseeds in distilled water. Sexual organs were produced in abundance when hempseed cultures were placed at a temperature of 20° C. Their development in living condition was studied under a water immersion lens. They were also killed and fixed in various stages of development using chromo-acetic acid sclution, Claussen's chromo-acetic acid (Claussen, 1908) and Raper's chromic acid formalin solution (Raper, 1936). The last-named solution gave the best results. The time for fixation varied from twelve to twenty-four hours. After fixation, the material was washed, dehydrated, and embedded in paraffin in the usual manner. Sections were cut 4–5 μ thick and were stained on the slide with crystal violet according to Gram's technique described by Couch (1932) and modified by Shanor (1937).

Observations on living material

The oogonia arise singly as terminal bodies on long slender branches of the hyphae. Sometimes intercalary oogonia are produced. The branches destined to form oogonia become densely filled with cytoplasm. The tip of the branch begins to swell by streaming in of the cytoplasm and this continues till it becomes rounded to form the young oogonium. The cytoplasm at this stage is more or less homogeneous and densely packed (Fig. 1 *a*).

Soon a central vacuole appears in the young oogonium (Fig. 1 *b*), which becomes cut off from the parent hypha by a transverse wall at its base,

Vol. XXIX, Parts I & II: issued 21 *May* 1946

leaving only a short neck (Fig. 1 *c*). Before the transverse wall is laid down, several antheridial initials, arising androgynously or diclinously, come to touch the oogonium, and antheridia of various sizes are cut off by transverse walls. The cytoplasm which enters the antheridium is not very dense. After delimitation the antheridia elongate and encircle the oogonium in an ellipsoidal curve.

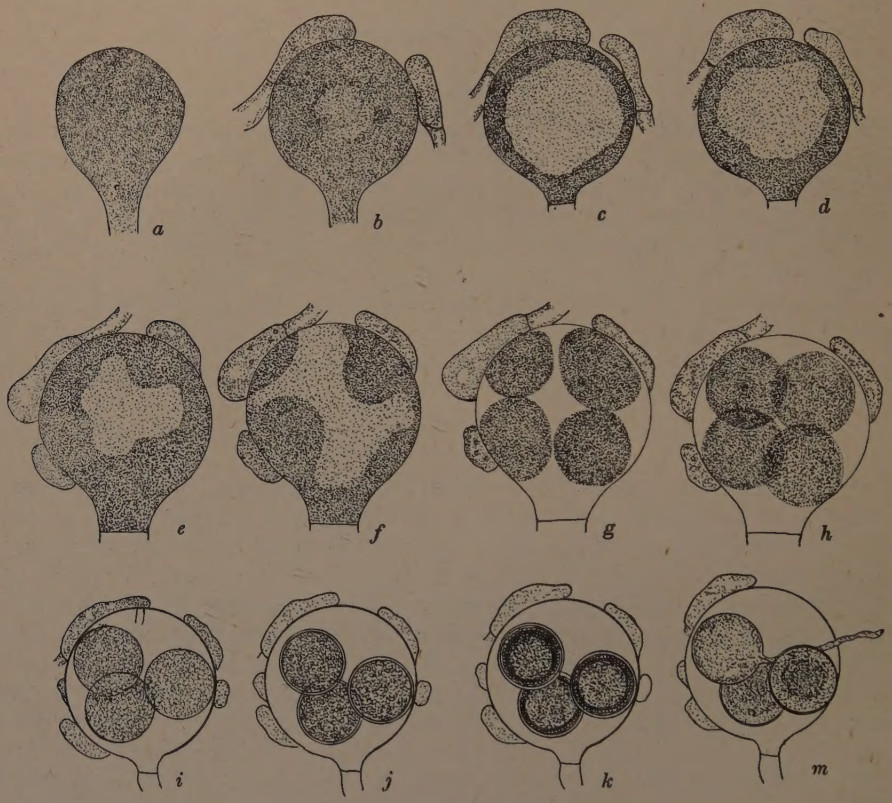


Fig. 1. Development of the oogonium, antheridium and oospore in living condition. × 1025. *a*, swelling of the tip of a hypha forming oogonium initial. *b*, appearance of a small vacuole in the centre and two antheridia attached on the wall. *c*, appearance of a transverse wall at the base and enlargement of the vacuole. *d*, the vacuole producing cleavages. *e*, cleavages more prominent showing heapings of the cytoplasm. *f*, cleavages still more marked and cytoplasm in the form of roughly conical masses. *g*, oogonium initials being formed. *h*, oospheres maturing. *i*, a fertilization tube has made entrance in the oogonium. *j*, young oospores with their wall thickened. *k*, thick-walled mature oospores with oil droplets formed. *m*, an oospore germinating in an oogonium. Others are still dormant.

The central vacuole enlarges considerably, pushing the cytoplasm towards the periphery (Fig. 1 *c*). It then sends out projections (Fig. 1 *d*, *e*) which by furrowing the cytoplasm separate it into definite conical masses (Fig. 1 *f*). The intervening cytoplasmic areas between these masses become thinner and thinner till they are ruptured by the extending cleavages. The separate masses of the cytoplasm swell up and begin to

round themselves to form oospheres (Fig. 1*g*), which, when fully grown, appear as perfectly spherical bodies (Fig. 1*h*). The number of oospheres ranges from one to ten; the oospheres measure 21·8–55·7 μ in diameter.

The coarse, granular appearance of the contents of the oospheres changes and oil globules begin to appear in the cytoplasm. Each oosphere has a thin membrane around it (Fig. 1*i*). Soon after this, fertilization tubes are sent into the oogonium by the antheridia but they are visible only for part of their length (Fig. 1*i*). The cytoplasm from the antheridia passes into these tubes but I could not see its migration into the oospheres.

Each oosphere now surrounds itself with a thin wall which gradually gets thicker and thicker till it becomes 3 μ thick. This is the oospore (Fig. 1*j*). Equal sized oil globules are formed and arranged in two or three rows about the periphery of the oospore giving a characteristic centric or subcentric arrangement (of oil droplets) (Fig. 1*k*).

During the process of oospore germination, which is rather rare and seems to be governed by some factors as yet not understood, the thick wall of the oospore gradually becomes thin. The oospore germinates *in situ*, after two days giving out a germ tube (Fig. 1*m*). The oil globules gradually disappear probably providing nourishment to the germinating oospore, in which a vacuole also appears. The germ tube elongates, comes out piercing the wall of the oogonium and gives rise to the mycelium. I did not observe the formation of sporangia containing spores on the short hyphae formed by the germination of oospores as observed by Trow (1899) in *Achlya americana* or by Weston (1918) in *Thraustotheca clavata.*

The antheridia remain attached to the oogonial wall until after the oospore germinates.

Effect of temperature on the production of sex organs

To find out the optimum temperature for the production of sex organs, the fungus growing on hempseeds was kept at various temperatures ranging from 5 to 30° C. It was observed that at 5° C. only a few oogonia appeared, some of which did not have antheridia. Cultures kept at 10 and 15° C. produced an abundance of oogonia but not all of them bore antheridia. At 20° C. the fungus produced numerous oogonia, all of which bore antheridia. The temperature of 25° C. had a retarding effect on the formation of antheridia, since only a few of the oogonia formed had antheridia. The high temperature of 30° C. completely inhibited the formation of sexual reproductive bodies.

Observations on fixed material

The young oogonium is filled with dense cytoplasm and contains many nuclei (Fig. 2*a*). The structure of the nuclei is similar to that of the nuclei in the vegetative hyphae. The nuclei are small, spherical, and contain a conspicuous central nucleolus from which a number of chromatin threads radiates.

In the next stage, a vacuole is seen in the centre of the cytoplasm, with

the nuclei shifted towards the periphery (Fig. 2 *b*). The antheridium also is multinucleate. At about this stage the basal wall cutting off the oogonium from its parent hypha is complete.

Fig. 2. Stages in the development of oospore in fixed material. × 1433 except *d* and *e* which are × 3000. *a*, section of a young oogonium showing nuclei in resting condition. *b*, section of an oogonium showing a small central vacuole and peripheral arrangement of the cytoplasm and the nuclei. Antheridia seen attached to the oogonium. *c*, section of an oogonium showing some nuclei in the process of degeneration. *d*, early prophase nucleus from an oogonium. *e*, late prophase nucleus showing the clustering of the chromatin into curious rod-like bodies. *f*, section of an oogonium and attached antheridia showing nuclei in metaphase. *g*, some of the daughter nuclei degenerating. *h*, section of an oogonium showing early stages in the cleavage of uninucleate egg origins. *i*, a later stage during the cleavage showing three separate oosphere initials. *j*, young oospheres with a single nucleus. *k*, an oosphere containing a female gamete nucleus in the centre and the male gamete nucleus towards the periphery. *m*, an oosphere showing male and female nuclei in contact. *n*, section of an oogonium showing multinucleate fertilization tube attached to an oosphere containing a fusion nucleus: two nucleoli are visible. *o*, a mature oospore containing a large fusion nucleus. *p*, a germinating oospore showing the nucleus in late prophase. Antheridia are still attached. *q*, a germinating oospore with a germ tube. Several nuclei are visible.

On account of the enlargement of the vacuole in the centre of the oogonium, the nuclei and the dense cytoplasm become limited to a

rather thin peripheral region. At a later stage, a number of nuclei are seen to be degenerating (Fig. 2*c*). The remaining nuclei then begin to divide. The oogonial nuclei enlarge considerably in early prophase (Fig. 2*d*). In late prophase the chromatin material becomes clustered around the nucleolus in the form of rod-like bodies (Fig. 2*e*). In the metaphase, nuclei show definite spindles, the longitudinal axes of which are nearly parallel to the wall of the oogonium. Some of the spindles may be seen to be surrounded by a nuclear membrane and are therefore intranuclear (Fig. 2*f*). Not all the stages from the prophase to the metaphase could be found. The chromosomes stain very darkly with gentian violet and are extremely small. They could not be counted with certainty but they are not less than eight. Simultaneously, the antheridia attached to the oogonium show nuclear divisions.

Following mitosis, most of the daughter nuclei degenerate, leaving only those which are to function as female nuclei (Fig. 2*g*). Eventually the contents of the oogonium are seen to be dividing up as the furrows advance from the vacuole (Fig. 2*h*). Each separate mass is uninucleate (Fig. 2*i*).

In the next stage, round oospheres lie within the oogonium and each has one nucleus in its centre (Fig. 2*j*).

The male gametic nucleus discharged from the fertilization tube lies near the periphery of the oosphere (Fig. 2*k*). It then moves towards the female gamete nucleus and comes to lie by its side (Fig. 2*m*).

In Fig. 2*n* a multinucleate fertilization tube from an antheridium is seen lying in contact with the oosphere, in the centre of which the male and the female nuclei are fusing. The membrane at the point of contact has been lost, and the fusion nucleus contains two nucleoli.

Finally the oospore is seen containing one nucleus which has enlarged considerably (Fig. 2*o*). The oospore nucleus, on germination, begins to divide. Fig. 2*p* shows the oospore nucleus in the late prophase. In the germinating oospore a germ tube is seen containing several nuclei (Fig. 2*q*).

The astral rays so prominently shown in *Leptolegnia caudata* by Couch (1932) and in *Thraustotheca clavata* by Shanor (1937), among other workers, were not seen in any stages of the development of sex organs.

Discussion

The results of the investigation are in general agreement with those of other workers who have studied the development of sexual organs in the Saprolegniaceae, and definitely establish the occurrence of fertilization in *Isoachlya anisospora* var. *indica*. *Isoachlya* is, therefore, another genus added to the list of Saprolegniaceae in which fertilization has been observed.

As regards the nuclear division in the oogonium and antheridium, Shanor (1937), in *Thraustotheca clavata*, found that mitosis in the antheridium begins after the division of the nucleus has progressed in the oogonium. Wolf (1938) reported that the division of the antheridial nucleus precedes that within the oogonium. The results obtained here are contrary to those of Shanor (1937) and Wolf (1938) but they agree with the findings of other investigators (Kasanowsky, 1911; Patterson, 1927, etc.) who have

reported that the nuclear division in the oogonium and antheridium occurs simultaneously.

There is only one nuclear division in the oogonium and antheridium of *Isoachlya anisospora* var. *indica*. Trow (1904) claimed that two nuclear divisions occur within the oogonium of *Achlya de Baryana* and probably also in *A. polyandra*. His view has been disputed by all other investigators who have shown that there is only one nuclear division within the oogonium or antheridium.

The chromosomes in the nuclei of water moulds are extremely small and cluster together so closely that it is very difficult, if not impossible, to make an exact count. Up to this time all workers have been able to give the approximate number in the species investigated by them, excepting Trow (1895) who reports the presence of one chromosome in *Saprolegnia dioica* and *S. mixta*.

Most of the workers have reported the presence of a centrosome and astral rays radiating from it. Patterson (1927), however, did not observe these rays in *Achlya colorata*. Raper (1936) reported the absence of astral radiations in *A. bisexualis* and Wolf (1938) failed to observe centrosomes and astral radiations in *A. flagellata*, in which they had been observed by Moreau and Moreau (1935). Höhnk (1935) reported the presence of astral radiations but no centrosome in *Saprolegnia ferax*. I, like Wolf, incline to the view that their absence is perhaps due to the difference in the methods of fixation and staining employed.

Summary

The general development and the cytology of the sexual organs of *Isoachlya anisospora* (de Bary) Coker var. *indica* is essentially the same as that of other members of the family Saprolegniaceae.

There is only one nuclear division in the oogonium and antheridium and it occurs simultaneously in both. The decrease in the number of nuclei is due to degeneration. No astral rays have been seen radiating from the nuclei. The number of chromosomes is not less than eight.

Fertilization has been shown to take place in *Isoachlya anisospora* var. *indica*.

I wish to express my warmest gratitude to Dr R. K. Saksena, University of Allahabad, for his advice and criticism throughout the course of the present study. My grateful thanks are here offered to Prof. S. R. Bose, Calcutta; Dr G. Watts Padwick and Dr B. B. Mundkur, New Delhi, for the generous loan of relevant literature and to Mr S. D. Garrett, Rothamsted Experimental Station, Harpenden, for kindly going through the manuscript.

REFERENCES

CLAUSSEN, P. (1908). Über Entwicklung und Befruchtung bei *Saprolegnia monoica*. *Festschr. dtsch. bot. Ges.* XXVI, 144–61.

COUCH, J. N. (1932). The development of the sexual organs in *Leptolegnia caudata*. *Amer. J. Bot.* XIX, 584–99.

HÖHNK, VON W. (1935). Zur cytologie der Oogon- und Entwicklung bei *Saprolegnia ferax* (Gruith) Thuret. *Naturwissenschaftlichen Verein zu Bremen*, XXIX, 308–23.

KASANOWSKY, V. (1911). *Aphanomyces laevis* de Bary. I. Entwicklung der sexual organe und Befruchtung. *Ber. dtsch. bot. Ges.* XXIX, 210–28.

MOREAU, F. & MME F. MOREAU (1935). Les phénomènes cytologiques du développement de l'œuf et de la fécondation chez les champignons du groupe des Saprolégniées. *C.R. Acad. Sci., Paris*, CCI, 1208–10.

PATTERSON, P. M. (1927). Fertilization and oogenesis in *Achlya colorata*. *J. Elisha Mitchell Sci. Soc.* XLIII, 108–23.

RAPER, J. R. (1936). Heterothallism and sterility in *Achlya* and observations on the cytology of *Achlya bisexualis*. *J. Elisha Mitchell Sci. Soc.* LII, 274–93.

SAKSENA, R. K. & BHARGAVA, K. S. (1944). A new variety of *Isoachlya anisospora* (de Bary) Coker. *Curr. Sci.* XIII, 79.

SHANOR, L. (1937). Observations on the development and cytology of the sexual organs of *Thraustotheca clavata* (de Bary) Humph. *J. Elisha Mitchell Sci. Soc.* LIII, 119–36.

TROW, A. H. (1895). The karyology of Saprolegnia. *Ann. Bot., Lond.*, IX, 609–52.

TROW, A. H. (1899). Observations on the biology and cytology of a new variety of *Achlya americana*. *Ann. Bot., Lond.*, XIII, 131–79.

TROW, A. H. (1904). On fertilization in the Saprolegnieae. *Ann. Bot., Lond.*, XVIII, 541–69.

WESTON, WM. H. (1918). The development of *Thraustotheca clavata*, a peculiar watermold. *Ann. Bot., Lond.*, XXXII, 155–73.

WOLF, F. T. (1938). Cytological observations on gametogenesis and fertilization in *Achlya flagellata*. *Mycologia*, XXX, 456–67.

(*Accepted for publication* 21 *March* 1946)

SIZE AND FORM IN AGARICS

By C. T. INGOLD, *Birkbeck College, University of London*

(With 3 Text-figures)

Introduction

On reading the second edition of D'Arcy Thompson's *Growth and Form*, it occurred to me that the concept of form tending to alter as a consequence of change of size, although it has been applied successfully to animals and green plants, has not been used in Fungi. Further it seemed that the agaric sporophore, because of its rather regular and geometric form, offered a good subject for testing the general thesis that form tends to vary with size, especially as the range is so great in Agaricaceae with *Marasmius Hudsonii* (pileus $\frac{1}{4}$ in. diam. × sporophore 2 in. high × stipe $\frac{1}{64}$ in. thick) at one extreme and *Psalliota villatica* ($14 \times 7\frac{1}{2} \times 3$ in.) at the other.

In the fruit-body of an agaric of the usual form the central stipe has, as Buller (1909) pointed out, two chief functions: the support of the cap, and the provision of a space below the pileus where air currents may have a reasonable opportunity of wafting the spores away before they reach the ground in the immediate vicinity of the sporophore.

Cap diameter and width of stipe

The weight of the cap in an agaric may be taken as roughly proportional to its volume. This weight acts vertically on the stipe and the strain is distributed over the area of the cross-section. If it is assumed that organisms are economical with their tissues, it might be expected that the stipe would tend to be of a thickness just sufficient to support the pileus with the rigidity necessary for the proper functioning of the sporophore.

In solid figures which vary in size but not in form, the volume varies as the cube of the linear dimensions (e.g. height or breadth), but the surface, and other two-dimensional features, vary as the square of these dimensions. Thus the volume, and, therefore, the weight, of the pileus varies as the cube of its diameter, but the cross-section of the stipe varies as the square of its width. These considerations would lead to the expectation that the sporophore would not remain constant in form with change of size but that, as compared with a central type, the smaller fruit-bodies would tend to have relatively slender stipes and the larger ones relatively thick ones.

To test this expectation the agarics listed in Ramsbottom's *Handbook of the Larger British Fungi* have been considered, omitting only species in which the stipe is lateral or wanting. The dimensions of the fruit-bodies, derived from an earlier handbook by W. G. Smith (1908), are given in the following form: e.g. *Amanita phalloides* $3\frac{1}{2} \times 4\frac{1}{2} \times \frac{5}{8}$. Smith remarks: 'The dimensions give the typical size in inches of well-developed examples as

found in Britain.... The numerals represent the diameter of the pileus, the height from the base of the stem to the top of the pileus, and the diameter of the stem near the middle.' The figures are clearly estimates and not objective determinations based on actual measurements of a large number of specimens of each species. It seems probable, however, that dealing with such large numbers (580 species) imperfections of the data may largely average out and the general pictures be not obscured.

In Fig. 1, with diameters of the pileus as abscissae and diameters of the stipe as ordinates, most of the species of Ramsbottom's *Handbook* are plotted, except for thirty very large ones which fall outside the area of the figure.

The average cap diameter for all the 580 species is 3·065 in. and the average stipe diameter 0·505 in. To make the arithmetic easier, a fruit-body having a cap 3 in. across and a stipe $\frac{1}{2}$ in. thick has been taken as the average type. The straight line in Fig. 1, drawn through the origin and through the point representing this 'average type', would be the graph of cap width plotted against stipe thickness if the *form* of the 'average type' were maintained irrespective of size. If the actual values plotted in Fig. 1 tended on the average to conform to this graph, they would be distributed

Table 1

Diameter of stipe (in.)	No. of species	Average pileus width
$\frac{1}{16}$	26	0·96
$\frac{1}{8}$	63	1·25
$\frac{3}{16}$	35	1·94
$\frac{1}{4}$	89	2·15
$\frac{3}{8}$	58	2·63
$\frac{1}{2}$	91	3·33
$\frac{3}{4}$	78	3·77
1	66	4·55

equally to left and right of this line throughout its length. This is not so. In the lower region the points are mainly to the right of the line and in the upper region to the left. It is clear that with changing size the *form* tends to alter and the smaller fruit-bodies have relatively slender stalks whilst the larger ones have relatively thick stalks.

In Ramsbottom's *Handbook* certain values for thickness of stipe are very commonly recorded. These are given in Table 1 together with the average pileus width for all those species listed as having each stipe diameter.

The values are also plotted in Fig. 2. In the same figure the straight line $y = 0{\cdot}16x$ is shown, y being the diameter of the stipe and x that of the pileus. It is the same line as that drawn in Fig. 1. The equation is derived from the dimensions of the 'average type' where $y = 0{\cdot}5$ and $x = 3{\cdot}0$. The curve in Fig. 2 corresponds to the equation $y = \sqrt{(0{\cdot}0092x^3)}$, and is the curve which might be expected if, as the volume (weight) of the cap varies, the diameter of the stipe is so adjusted that the weight acting on unit cross-section remains the same. This curve also is derived from the 'average type' thus:

$$\frac{y^2}{x^3} = \frac{0{\cdot}5^2}{3{\cdot}0^3} \quad \text{or} \quad y = \sqrt{(0{\cdot}0092x^3)}.$$

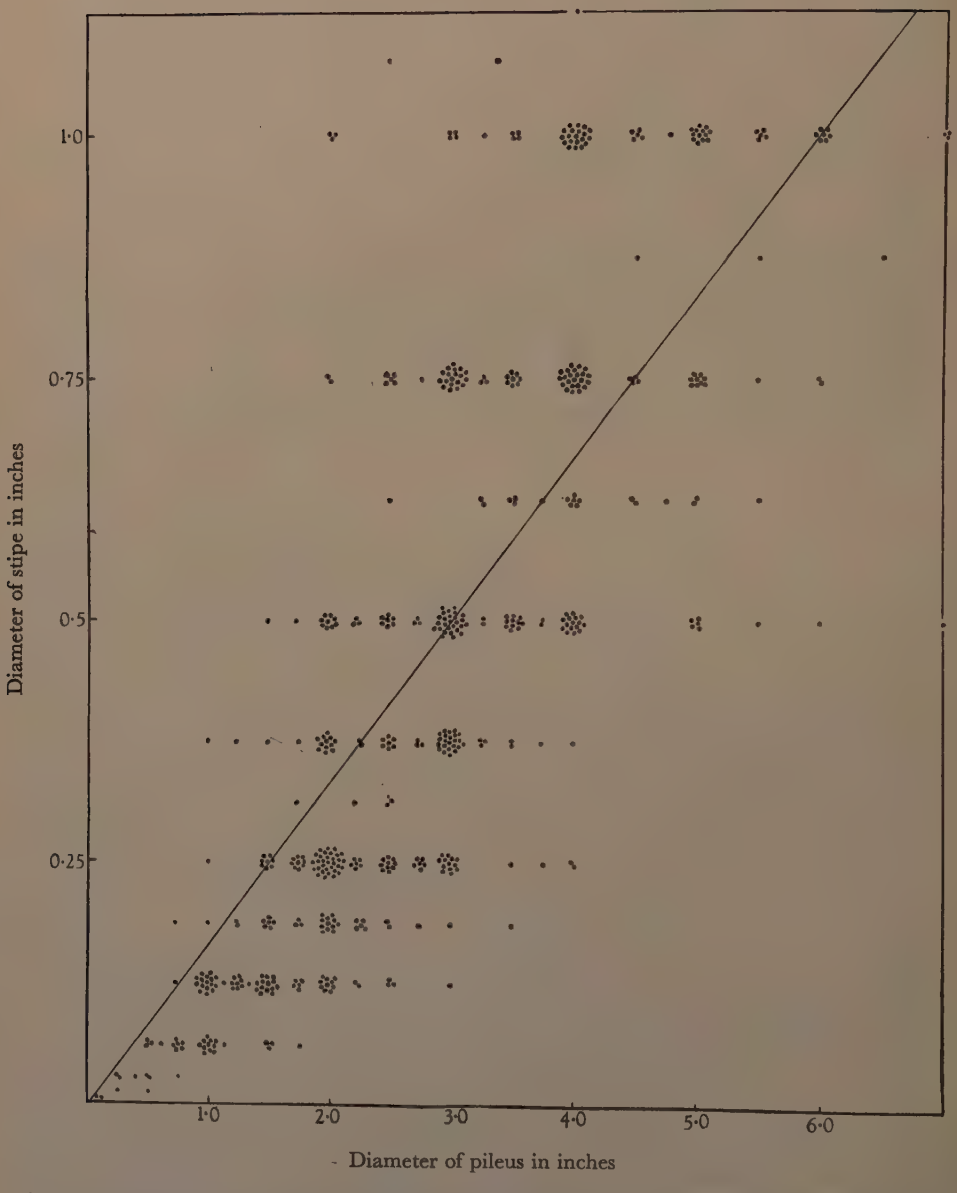


Fig. 1. Diameter of cap plotted against diameter of stipe. Each dot represents a single species. Where two or more points should be in the same position, they have been grouped close together around this position.

Clearly this equation and $y = 0{\cdot}1\dot{6}x$ have two solutions in common, namely, $x = 0, y = 0$ and $x = 3{\cdot}0, y = 0{\cdot}5$.

An examination of Fig. 2 shows that the actual points do not conform with the straight line, but show quite a striking approximation to the curve.

It seems, therefore, that size and form are interrelated in agarics as in other organisms. Perhaps if a substantial body of objective data were

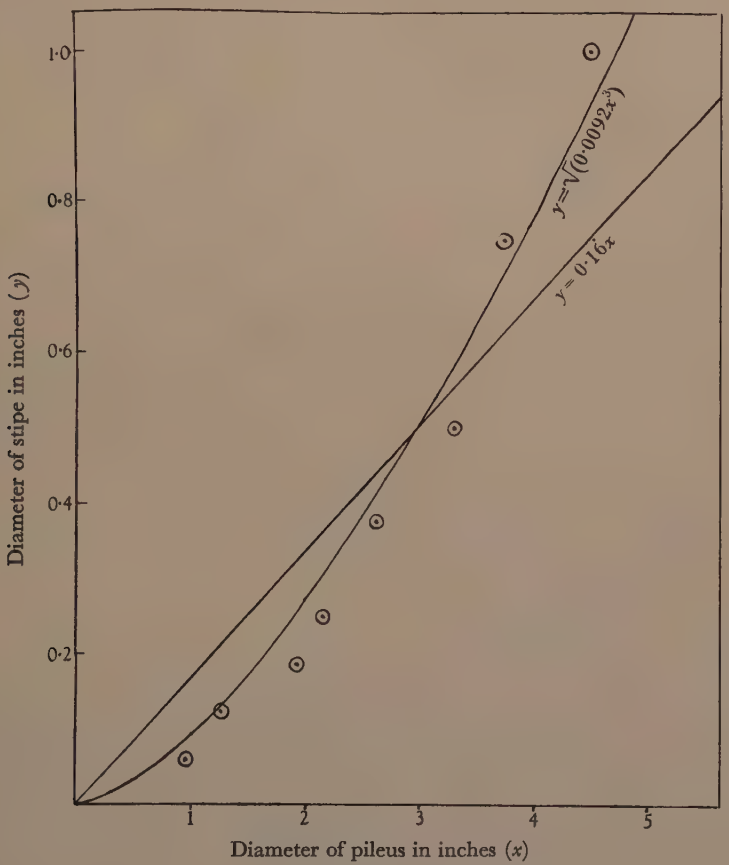


Fig. 2. For explanation see text.

available the relationship of size and form might be even more clearly demonstrable. In this connexion it would be most interesting to collect for a large number of species, and for a large number of individuals of each species, data of pileus weight and the corresponding area of cross-section of the stipe.

Cap diameter and height of sporophore

The height of an agaric is mainly due to the length of the stipe which provides a space between the underside of the pileus and the ground so that

spores in their downward course have a good chance of being blown away before they reach the ground in the immediate vicinity of the fruit-body. With the development of larger and smaller types of agarics it might be expected that the general form would tend to be preserved. However, in the larger types the interval between cap and substratum would become greater than necessary and it might be expected that mutants having shorter stalks, and thereby economizing tissue, would tend to survive in the struggle for existence. On the other hand, in the smaller types, if the form remained unaltered, the interval between the pileus and the ground might be reduced too far for efficient dispersal and mutant types with longer stalks would tend to be selected. Thus we might expect that the larger

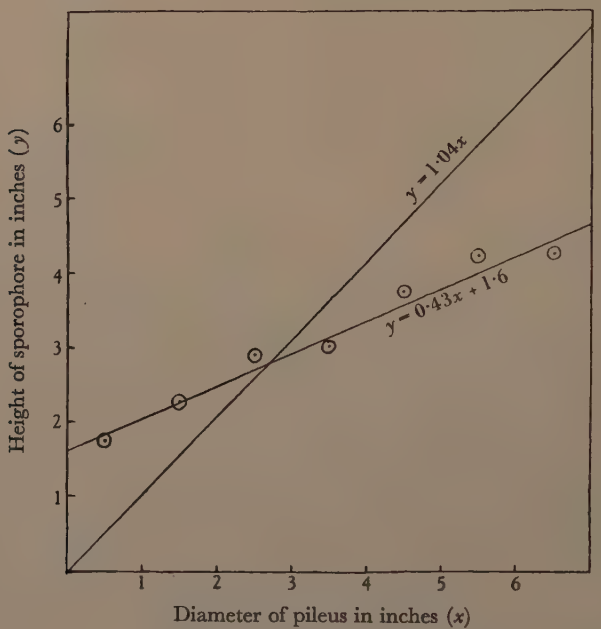


Fig. 3. For explanation see text.

sporophores would tend to be relatively short and the smaller ones relatively tall.

In Table 2 the species are divided up into size groups based on pileus width. The number in each group is shown with the average height for members of that group. These averages are plotted in Fig. 3 together with a straight line drawn through the origin and through the point representing the 'average type' (pileus diameter 3 in., height 3·1 in.). This line ($y = 1{\cdot}04x$) would represent variation of height with pileus diameter if the form remained unaltered irrespective of size. Clearly the points depart greatly from this line. Indeed, they appear to approximate to a straight line of the type $y = mx + c$ and that drawn in Fig. 3 is $y = 0{\cdot}43x + 1{\cdot}6$. The value (0·43) of the constant 'm' is quite arbitrary and is selected merely to

Table 2

Pileus diameter Size group (in.)	No. of species in group	Average height (in.)
0–<1	22	1·75
1–<2	101	2·26
2–<3	147	2·89
3–<4	140	3·01
4–<5	85	3·73
5–<6	40	4·21
6–<7	15	4·25

give as close a fit as possible with the plotted points. The constant '*c*', which appears to have a value of about 1·6 in., may be pictured as the average minimum height of fruit-body for efficient spore dispersal.

Summary

Small agarics tend to be relatively tall and have relatively thin stalks, and large agarics tend to be relatively short and have relatively thick stalks as compared with an 'average type'. It is suggested that these tendencies for form to alter with size follow from simple mechanical and functional considerations.

REFERENCES

Buller, A. H. R. (1909). *Researches on Fungi*, 1. London.
Ramsbottom, J. (1923). *A Handbook of the Larger British Fungi*. London.
Smith, W. G. (1908). *Synopsis of the British Basidiomycetes*. London.
Thompson, D'Arcy W. (1942). *On Growth and Form*. Cambridge.

(*Accepted for publication* 2 *April* 1946)

A STUDY OF VIOLET ROOT ROT

FACTORS AFFECTING PRODUCTION AND GROWTH OF MYCELIAL STRANDS IN *HELICOBASIDIUM PURPUREUM* PAT.

By S. D. GARRETT, *Rothamsted Experimental Station, Harpenden*

(With Plates V and VI)

The symptoms of Violet Root Rot are so pronounced, and so obviously attributable to infection by a root-infecting fungus, that it would be difficult to mistake this disease for any other. It was therefore possible for Duggar (1915), in his review of early work on violet root rot, to cite an unmistakable description of this disease on saffron (*Crocus sativus*) published by Duhamel (1728). The causal fungus was named *Sclerotium Crocorum* by Persoon (1801), and was later transferred by De Candolle (1815) to his new genus, *Rhizoctonia*, which included two species, *R. Crocorum* on crocus and other hosts, and *R. Medicaginis* on lucerne. Later, after a thorough morphological study of violet root rot in a number of hosts, the brothers L. and C. Tulasne (1851) reduced De Candolle's two species, *R. Crocorum* and *R. Medicaginis*, to a single species, which they named *R. violacea*. Duggar (1915) confirmed the Tulasnes's conclusions, but pointed out that their new specific epithet *violacea* contravened the Rules of Nomenclature, and that the correct name was *R. Crocorum* (Pers.) DC. As Buddin and Wakefield (1924) comment: 'It is unfortunate that the name is less descriptive than *R. violacea*, and is particularly inappropriate in this country where the crocus disease is unknown.' It only remains to add that as, according to the International Rules of Botanical Nomenclature, fungus names date from the publication by Fries (1823) of the *Systema Mycologicum*, the correct citation is now *R. Crocorum* Fr. (British Mycological Society, 1944).

More than a century of search for the perfect stage of *R. Crocorum* was ended by its discovery in a Basidiomycete, *Helicobasidium purpureum* Pat., by Buddin and Wakefield (1927). They found fructifications of *H. purpureum* in very close association with violet root rot in a number of localities and on three different hosts, red clover, dog's mercury (*Mercurialis perennis*), and nettle (*Urtica dioica*). Morphological characters of the mycelium of *Helicobasidium purpureum* appeared identical with those of *Rhizoctonia Crocorum* both on the host and in pure culture, and conidia of the type *Tuberculina* were commonly produced in cultures of both forms. Finally, typical violet root rot was produced by inoculation with mycelial cultures derived from three distinct basidiospore isolates of *Helicobasidium purpureum*. Additional evidence in support of the connexion between *Helicobasidium purpureum* and *Rhizoctonia Crocorum* was published by Buddin and Wakefield (1929) two years later.

During the course of the investigations to be reported here, still further evidence in confirmation of Buddin and Wakefield's conclusion was incidentally obtained. In mid-April 1945, fertile fructifications of *Helicobasidium purpureum* on old cut stems of the previous season were found in boxes of red clover inoculated with a culture of *Rhizoctonia Crocorum* (originally isolated by Dr R. Hull from a sugar beet in Lincolnshire) in March 1944. The identification of *Helicobasidium purpureum* was confirmed by Miss Wakefield.

In this country at the present time, violet root rot is of economic importance chiefly as a disease of sugar beet (Hull & Wilson, 1946), though it also affects other field crops, such as clover and other legumes, mangold, carrot and potato, and a variety of more strictly horticultural crops, such as asparagus and seakale (Moore, 1943). The need for further study of factors influencing infection by the violet root rot fungus was emphasized by Buddin and Wakefield (1927) in their statement: 'It is obvious that one of the most pressing needs is for more exact information as to the conditions under which *Rhizoctonia* will infect living roots.'

Development of the fungus on the host

In plants inoculated in the glasshouse by means of sclerotia buried in the potting soil, a preliminary to infection can be noted three or four weeks after inoculation, in the form of red epiphytic mycelial strands attached at occasional points to the roots. After some eight weeks, infection cushions can usually be found. Prillieux (1891) was the first to point out that these infection cushions, or 'corps miliaires' as he called them, constituted the chief mode of tissue invasion by the fungus. Prillieux's view that these bodies were simply infection cushions was upheld by Duggar (1915) and Faris (1921). The infection cushions are connected at their apices with the mycelial web extending over the host epidermis, and are at least partially sunk into the cortical tissue; their development on young potato stems and tubers has been described by Faris (1921). It is unfortunate that, on account of their morphological resemblance to small sclerotia, these bodies have been termed microsclerotia by some writers: functionally, they are very different from the true sclerotia, which are produced towards the end of parasitic activity, and constitute the resting stage of the fungus.

The true sclerotium was first described by Duhamel (1728) as a 'tubercule', which he thought to be the fructification of a fungus related to the truffles. The sclerotia vary greatly in size, though usually not exceeding 5 mm. in length on the host plant; in form they tend to be elongated, and often with a suggestion of compound structure. Sclerotia may be produced on the mycelial web or strands, as described by Duggar (1915) and Faris (1921), but on sugar beet and clover they are most frequently formed around small lateral roots, along which they may even be strung like beads on a thread. The vascular elements of the rootlet can then be distinguished when examining a section of the sclerotium under the microscope. During the present investigation, isolations of *Helicobasidium purpureum* have been obtained without difficulty by surface sterilizing sclerotia for approximately

a minute in 1 : 1000 mercuric chloride in 10% alcohol, washing in sterile water, and plating out on malt-extract agar.

In plate cultures of the fungus on a suitable agar, such as meat-malt extract agar of twice normal concentration, sclerotia begin to be discernible some four weeks (at 25° C.) after inoculation of the plates, and they continue to develop visibly for at least another month. A conspicuous feature of these sclerotia is their persistent golden-brown mantle of setae, which can be seen in Plate X*b* of the paper by Buddin and Wakefield (1924). Sclerotia formed in plate culture are very variable in size, and compound sclerotia are common, especially around the site of the inoculum piece.

Methods

Handling of soils and soil containers. Stocks of soil for experimental work are passed through a $\frac{1}{4}$ in. sieve, then air-dried and passed through a fine sieve (14-mesh per in.). The clods of soil are crumbled by hand, but not mechanically crushed, because heavy crushing breaks down some of the soil crumbs into their constituent particles, and so gives an abnormally fine tilth (Russell, 1938). After thorough mixing of the whole stock of air-dried soil, saturation capacity is determined by the perforated box method (Keen & Raczkowski, 1921). For work with moisture contents down to 40% saturation, the calculated amount of water can be added from a measuring cylinder to the surface of the air-dry soil in its individual container. For moisture contents of 30% saturation or less, it is advisable to mix soil and water by hand before filling the containers, but after this procedure it is difficult to get as close or as uniform a packing of the moist soil as when water is added to the air-dry soil already settled in the container. For this reason, a moisture content of 40% saturation was the lowest studied in the present investigation. A range of soils varying in texture can be obtained by progressive dilution of the soil with sand. A range of *p*H values is achieved by treating portions of soil with suitable amounts of sulphuric acid or calcium hydroxide. After treatment, the moist soils are incubated in 12 in. unglazed pots in the glasshouse for at least a month, for equilibrium with the reagents to be reached as far as possible; the treated soils are then air-dried for use in the laboratory. Wherever possible, factorial designs have been used for these experiments, and soil containers have been arranged in randomized blocks on the laboratory table.

Measuring growth of mycelial strands. The activity of root-infecting fungi on the host roots and in the soil has been investigated by the glass-tumbler method in two ways: (i) seedling plants have been used by Garrett (1936) for *Ophiobolus graminis*, by Shen (1940) for *Fusarium culmorum*, and by Samuel and Garrett (1945) for *Plasmodiophora Brassicae*; (ii) Rossi-Cholodny slides have been used by Blair (1943) for *Rhizoctonia Solani*. For the present investigation on *Helicobasidium purpureum*, Rossi-Cholodny slides were found satisfactory, but seedlings proved unsuitable; mycelial growth of *Helicobasidium purpureum* is slower than that of *Ophiobolus graminis*, and even four weeks after sowing and inoculating seed, the epiphytic mycelial

strands of *Helicobasidium purpureum* are still only lightly attached at occasional points to the seedling tap-root. A satisfactory alternative to seedlings was eventually found in a plant-storage organ, the potato tuber, and this was used in preference to the Rossi-Cholodny slide for the majority of experiments.

The potato-tuber method, as finally adopted, was as follows. The fungus was grown on a suitable agar medium, and when colonies were at an optimum age for production of inoculum, disks were cut all round, equidistant from the centre, with an 8·5 mm. cork borer. With the same cork borer, an approximately hemispherical piece of tissue was removed from the centre of the most even and regular face of the tuber, and the agar culture disk placed in the wound. The soil container, a 2 lb. glass jam jar, of diameter 8 cm. and height 14 cm., received first one-third of the total weight of dry soil, which was shaken down and then brought to the calculated moisture content. The tuber was placed, inoculum downwards, on the moist soil, and the remaining two-thirds of dry soil was added, shaken down and brought to correct moisture content. When different soils were compared, weights of soil were so adjusted as to give equal volumes; the moist soils, with tubers, filled the jars to a height of 8–9 cm. The experiments were allowed to run for some seven weeks in the laboratory (mean temperature 15–20° C.), soil moisture content being made up weekly on a box balance. Tubers were then washed out of the jars as gently as possible, so as not to dislodge the mycelial strands, and preserved in formalin for recording.

Growth of mycelial strands from the inoculum disk was measured under the binocular dissecting microscope, with magnification 20×; a dozen headed pins were used as combined dissecting needles and markers. For some distance out from the inoculum disk, the growth of the red mycelial strands could be followed with ease, sometimes even by the naked eye (Pl. V, fig. 1), but further out the strands became progressively thinner and paler in colour, so that a dissecting needle was used to tease them up from the tuber surface for better visibility. When the tip of the strand had been determined, its position was marked by sticking the needle into the tuber. When the tips of all the longer strands had been marked, the distance of the farthest from the inoculum disk was measured over the curved surface of the tuber by means of a paper scale marked in millimetres, and recorded as *maximum growth of mycelial strands*, for each tuber.

The type of mycelial disk inoculum was found to be of great importance, but its specification is best reserved for the next section. Wounding with the cork borer was subsequently found not to be necessary for production and growth of mycelial strands over the surface of the tuber, but was continued in order to protect the inoculum disk from possible desiccation immediately after covering the inoculated tuber with dry soil, when filling up the jar. Tubers of the potato varieties King Edward or Majestic were used according to convenience in all experiments; in a preliminary trial, there was no significant difference in extent of mycelial strand growth over tubers of these two varieties. Relatively young tubers were best for these experiments, owing to their lesser liability to bacterial rotting, especially in

soils of high moisture content, and their slower rate of sprouting in the jars. The sprouting of older tubers was not a serious disadvantage for the limited duration of these tests, however, because the incipient leaves on the sprouts failed to develop under laboratory conditions. A third disadvantage of old tubers, and one greater than either of these, was that resistance of the tuber to infection appeared to increase with storage age, as shown by a survey of all the experimental data. With increasing storage age of the tuber, from mid-August soon after digging (the earliest tested) to mid-April of the following year (the latest tested), distance grown by mycelial strands and frequency of infection cushions (Pl. V, fig. 2) both declined. Development of a progressive rot, in tubers infected by *Helicobasidium purpureum* while still attached to the parent plant, has been described by Faris (1921); an example of such progressive infection is illustrated in Pl. VI, fig. 1, showing two tubers collected from an infected potato crop. In Fig. 2 of Pl. VI is shown, for comparison, another potato tuber, also collected from an infected field, on which epiphytic strands of *Helicobasidium purpureum* are well developed, but unaccompanied by infection cushions. Of interest in this connexion is a statement made by a Northamptonshire farmer that he had clamped some potatoes which were covered with a brownish web of the violet root rot fungus, but were otherwise sound, and that when he opened the clamp in the following March, the potatoes, though still bearing the mycelium of the fungus, had not rotted.

Experiments with potato tubers

The experiments with tubers are concerned first with the effect of type of inoculum upon production and growth of mycelial strands, and secondly with the effect of soil conditions upon such growth.

Experiment I. In a preliminary experiment, wounded potato tubers had been inoculated with mycelial agar disks cut from the growing margin of the fungus colony, but growth of strands was either poor and irregular, or lacking altogether. It was therefore decided to test marginal disks against submarginal disks taken from the same colonies; the submarginal disks were cut with the same cork borer immediately inside the ring of marginal disks. The different agar media employed were all variations of the meat-malt extract agar found by Buddin and Wakefield (1924) to be the best of any that they tested for growth of this fungus. The standard formula taken for this agar was as follows: Lemco beef extract 10 g., malt extract 30 g., agar 20 g., and distilled water 1 l. Four concentrations—nil, normal, twice and thrice normal—of each constituent, meat and malt, were tested in all possible combinations with the four concentrations of the other constituent (except that the 0 meat-0 malt combination was omitted). Variation in growth rate of the fungus on these different meat-malt agars, as shown by mean colony diameter of triplicate plates after twenty-four days at 25° C., is shown in Table 1.

Most rapid growth of *Helicobasidium purpureum* was made on 1 meat-1 malt agar, i.e. that prepared according to the standard formula, and growth fell off somewhat with higher concentrations of meat and malt.

After twenty-six days' growth of the colonies at 25° C., five marginal and five submarginal inoculum disks were cut from a plate of each type of agar, and inoculated into potato tubers, variety King Edward, which were then buried in jars of a mixture of 1 vol. Harpenden allotment soil : 3 vol. sand, brought to a moisture content of 60% saturation. The experiment was set up on 8 February 1945, and was harvested after sixty days (mean laboratory temperature 20° C.): the results are given in Table 2.

Table 1. *Mean diameter of* Helicobasidium purpureum *colonies in mm.*

		Conc. of malt in agar			
		0	1	2	3
Conc. of meat in agar	0	—	41	51	51
	1	28	59	57	52
	2	33	55	54	52
	3	30	45	46	45

Table 2. *Maximum growth of mycelial strands in mm.*

		Marginal disks Conc. of malt in agar				Submarginal disks Conc. of malt in agar			
		0	1	2	3	0	1	2	3
Conc. of meat in agar	0	—	0	3	7	—	21	23	27
	1	0	1	8	7	0	5	22	33
	2	0	5	8	8	0	10	24	27
	3	0	11	12	15	0	19	36	29

The most striking feature of these results is the greater extent of strand growth from the submarginal disks. Also noteworthy is the complete absence of strand growth from disks of all the 0-malt agars. With a single exception, 3 meat-3 malt agar, strand growth increased with increasing concentration of malt in the agar. Optimal concentrations of meat and malt for production of strands were, however, supra-optimal for colony growth on the original agar plates (Table 1).

Experiment II. In this experiment, an explanation was sought for the better production and growth of mycelial strands from submarginal than from marginal inoculum disks. It seemed likely that in the marginal disks a higher proportion of the nutrients in the agar would still be outside the hyphae, and therefore available for assimilation by other soil micro-organisms in competition with *Helicobasidium purpureum*, after inoculation of the tubers. To test this possibility, marginal inoculum disks were cut from colonies on 2 meat-2 malt agar, three weeks old at 25° C., and treated in four different ways:

A. Used at once for inoculation of tubers.

B. Incubated in moist sterile Petri dishes at 25° C. for one week.

C. Contaminated with soil suspension, and incubated in moist Petri dishes at 25° C. for one week.

D. Cut, but left in position in original agar plates for one week at 25° C.

Ten potato tubers, variety Majestic, were inoculated with disks from each series, and buried in the 1 soil : 3 sand mixture, adjusted to a moisture

content of 60 % saturation. Tubers were inoculated with disks of series A on 7 August 1945 and with those of the other three series one week later. Maximum strand growth was measured after seven weeks (mean laboratory temperature 17° C.) in all series, and results are given in Table 3.

Table 3. *Maximum growth of mycelial strands in mm.*

A.	Disks used at once	28 ± 2·0
B.	Disks incubated sterile	27 ± 3·4
C.	Disks incubated unsterile	21 ± 2·8
D.	Disks cut, but left in position	38 ± 2·3

It is evident that incubation of the marginal inoculum disks under moist sterile conditions for one week has not made them any more effective as inoculum than the same disks used immediately for inoculation of the tubers. Assimilation by other soil micro-organisms of the unconsumed nutrients in the marginal disks during the first week after inoculation cannot therefore be held responsible for the poorer strand growth from such disks than from submarginal disks. Although growth from the disks of series C, incubated unsterile for a week, was slightly less than that from disks of series A and B, this reduction has not reached the 5 % level of significance (by the *t* test). This rather unexpected result seems at first difficult to explain, because mycelium in the marginal disks cut out and incubated sterile for one week at 25° C. would be almost identical in age with that in submarginal disks cut out one week later (for the reason that in one week at 25° C. the advance of the colony equals the diameter of the disk, 8·5 mm.: the incubation period of seven days was chosen for this reason).

The clue to this problem was afforded by observation of visible changes in the disks of series B and D after the week's incubation. Those of series D, left in their original positions after cutting, showed a much greater development of mycelium, as judged both by density of colour and by observation under the microscope, than those of series B, incubated separately in moist sterile dishes. Moreover, the disks of series D had to be recut at the end of the week's incubation, as they had been joined up again with the parent colony by renewed growth of mycelium. It is suggested, therefore, (i) that the greater effectiveness as inoculum of the disks of series D, and of submarginal disks in general, is due to a greater density of mycelium in such disks, (ii) that the greater density of mycelium in such disks is due not only to development of mycelium already within the disk area, but also to continued invasion of the disk area by hyphae from outside. This second conclusion may be stated in more general terms, as follows: *during one week's growth of a colony of* Helicobasidium purpureum *at 25° C., the original marginal zone of the colony is progressively invaded by hyphae from the submarginal zone.* The conclusion that development of mycelium within the marginal zone of a colony is due to infiltration of new hyphae from the submarginal zone, as well as to branching of the original hyphae within the marginal zone, is probably applicable to growth of fungus colonies in general.

One further comment is necessary on these results; the greater extent of

strand growth, both from marginal and submarginal disks, in this experiment than in Exp. I (Table 2) is to be ascribed to the shorter storage period of the tubers.

Experiment III. In spite of the employment of submarginal inoculum disks of 2 meat-2 malt agar, production and growth of mycelial strands in some subsequent experiments was still very variable. Several experiments on the effect of soil conditions on growth of mycelial strands were rendered worthless by the fact that many of the inoculum disks failed to produce any strands at all. In an endeavour to overcome this difficulty, a comparison was made between submarginal inoculum disks from plates poured at the normal depth of 3 mm. agar (20 ml. per plate) and at twice that depth (40 ml. per plate); a further comparison, between 2 meat-2 malt and 3 meat-3 malt agars was incorporated in this experiment. The plates were incubated after inoculation for four weeks at 25° C., and for a further week in the laboratory (mean temperature 19·5° C.). Ten tubers, variety King Edward, were inoculated on 5 April 1945 with each of the four types of submarginal inoculum disks, and buried in the same type of soil as in the previous experiments. Maximum growth of mycelial strands after nine weeks (mean laboratory temperature 17° C.) is recorded in Table 4.

Table 4. *Maximum growth of mycelial strands in mm.*

	Agar disks 3 mm. deep	Agar disks 6 mm. deep
2 meat-2 malt agar	5±1·5	22±4·3
3 meat-3 malt agar	15±3·5	27±4·0

The increase in maximum strand growth resulting from a double depth of agar is highly significant on the 2 meat-2 malt agar, but just fails to attain significance (by the *t* test) on the 3 meat-3 malt agar. Increase in strand growth with increase in nutrient concentration of the agar is significant only with the disks of shallow agar.

Experiment IV. It so happened that tubers for this experiment were inoculated one week later with submarginal disks taken from the same batch of plates as that used for Experiment III; the 2 meat-2 malt agar plates poured at double depth were selected for provision of inoculum. In all, ninety-six tubers, variety King Edward, were inoculated and kept for eight weeks (mean laboratory temperature 16° C.) in acid-treated (*p*H 5·5), untreated (*p*H 7·6) and limed (*p*H 8·0) Harpenden allotment soils at moisture contents of 40 and 60% saturation (sixteen tubers for each series). Results of this experiment were extremely irregular and unsatisfactory; only 23/96 inoculum disks gave any strand growth at all, and only 18/96 gave a strand growth exceeding 10 mm. In the soils at 40% saturation only 8/48 disks gave any growth, as against 15/48 disks for the soils at 60% saturation, but the growth failures certainly could not be attributed to unfavourable soil conditions as the principal cause.

The most obvious difference between this experiment and the preceding one, in which better strand growth had been obtained, was that the plates for inoculation had been kept for one week longer in the laboratory at a

mean temperature of 19° C. The decreasing potency of the disks for strand production might therefore have been due to (i) increase of one week in age, (ii) transfer from the 25° C. incubator to a cupboard at laboratory temperature. A survey of all available experimental data showed that the unsatisfactory results from a number of experiments could be completely explained by the fact that plates for inoculum had been incubated for four weeks at 25° C., and then for a further one or two weeks in a cupboard. No evidence was available to show, however, whether the unsatisfactory behaviour of inoculum disks from these plates was to be attributed to increased age of the colony, or to some deleterious effect of the transfer from the incubator at 25° C. to a cupboard at laboratory temperature. It was decided to test first the effect of colony ageing at a continuous temperature of 25° C.

Experiment V. In this experiment, plates were poured at normal (3 mm.) and double (6 mm.) depth with three types of agar, 0 meat-2 malt, 2 meat-2 malt, and 3 meat-2 malt, and inoculated with *Helicobasidium purpureum* at such times that colonies of ages three, five and six weeks at a continuous temperature of 25° C. would be available for the cutting of submarginal inoculum disks on the same day. On 29 August 1945 ten tubers, variety Majestic, were inoculated with each type of inoculum disk; there were disks of three agars, of two depths and of three ages, in the eighteen possible combinations, making 180 inoculated tubers altogether. The same 1 soil : 3 sand mixture at 60% saturation was used as in previous experiments. Maximum strand growth after seven weeks (mean laboratory temperature 16° C.) is given in Table 5.

Table 5. *Maximum growth of mycelial strands in mm.*

Type and depth of agar	Age of colonies (weeks)		
	3	5	6
0 meat, shallow	41	43	26
0 meat, deep	40	47	48
2 meat, shallow	38	28	28
2 meat, deep	40	43	43
3 meat, shallow	32	33	25
3 meat, deep	40	45	44

Table 5 shows that although there is a decrease in maximum strand growth from the shallow inoculum disks taken from 6-week colonies, and even from those from 5-week colonies on the 2-meat agar, there is no such decrease in strand growth with increasing age of colony from the deep disks, and so these results are quite inadequate to account for those of Experiment IV. The observed decrease in strand growth from disks taken from progressively older colonies on shallow agar is probably due to production of staling substances by the ageing colony. Thus Brown (1923) showed that colonies of *Sphaeropsis malorum* and of species of a *Fusarium* staled more rapidly on shallow than on deep layers of agar, presumably because of the greater dilution of staling substances under the latter conditions.

The general high level of figures for strand growth in Table 5 is to be

attributed to the fact that tubers were still in the early stage of their storage life.

Experiment VI. As ageing of colonies at a continuous temperature of 25° C. was shown in the last experiment to be inadequate to explain the unsatisfactory behaviour of inoculum disks in Experiments III and IV, it seemed that this must be attributed to the taking of inoculum plates out of the incubator at 25° C., and holding them for a further one and two weeks, respectively, at laboratory temperature. To test this conclusion, a number of plates of 2 meat-2 malt agar, poured at normal depth (3 mm.), were inoculated with *Helicobasidium purpureum* and kept in the 25° C. incubator for three weeks. Half the plates were then left in the incubator for a further two weeks, and half were kept for the same period in a dark cupboard, the temperature of which varied from 13° to 19° with a mean of 16·5° C. during the first week, and from 14° to 19° with a mean of 16·5° C. during the second week. Forty tubers, variety Majestic, were then inoculated with disks from these plates on 18 December 1945, and buried in the 1 soil : 3 sand mixture at 60% saturation. Maximum strand growth after seven weeks (mean laboratory temperature 15° C.) was $22 \pm 1{\cdot}9$ mm. in the series inoculated with disks from plates held continuously at 25° C., and $7 \pm 2{\cdot}4$ mm. in the other series. Evidently, therefore, the unsatisfactory behaviour of the inoculum disks in previous experiments was due not merely to ageing of the colonies, but to their transfer from the incubator at 25° C. to a cupboard at laboratory temperature for one or two weeks prior to cutting out of disks. Further research is required for elucidation of the mechanism of this effect.

In the meantime, it can be concluded from the results of these six experiments that, to secure best growth of mycelial strands, tubers should be in the early part of their storage life, and submarginal inoculum disks should be cut from plates held continuously at 25° C., preferably not more than three and a half weeks old. If these conditions are fulfilled, a 2 malt agar with any concentration of meat is adequate, but pouring of plates at double depth (6 mm.) is recommended as an additional safeguard, especially with older tubers.

Experiment VII. Having disposed of the difficulties described, it seemed possible to set up an experiment to determine the effect of soil conditions upon rate of growth of mycelial strands, with greater prospect of success. The experiment, of factorial design, comprised the eighteen possible combinations of three soil reactions, two textures, and three moisture contents. To secure soil of three different reactions, portions of moist Harpenden allotment soil were treated with 1·5% sulphuric acid, and with 0·25% calcium hydroxide, respectively; the treated soils, together with a third untreated portion, were incubated moist in 12 in. porous pots for eight weeks, and then air-dried and passed through the 14-mesh sieve. Two different soil textures were obtained by diluting part of each soil with three times its own volume of sand. Submarginal inoculum disks were cut from colonies on 2 meat-2 malt agar poured at double depth (6 mm.) and incubated at 25° C. for twenty-three days, and ten Majestic tubers were inoculated for each of the eighteen soil series on 10 September 1945;

optimum inoculum was therefore combined with the provision of tubers still in the early part of their storage life. Maximum strand growth (Table 6) was measured after a period of six weeks only, so that growth should be limited only by soil conditions, and not by reserves of the inoculum. Mean laboratory temperature during this period was 17° C.

Table 6. *Maximum growth of mycelial strands in mm.*

	Acid-treated soil	Untreated soil	Limed soil
Whole soil, 40% saturation	12	20	27
Whole soil, 60% saturation	17	23	27
Whole soil, 80% saturation	23	18	19
Soil/sand, 40% saturation	36	30	30
Soil/sand, 60% saturation	34	32	32
Soil/sand, 80% saturation	29	34	27
	*p*H values of soils		
Whole soil, start of exp.	5·1	7·4	7·7
Whole soil, end of exp.	5·1	7·3	7·6
Soil/sand, start of exp.	5·1	7·5	7·8
Soil/sand, end of exp.	5·0	7·7	7·9

The most outstanding feature of these results is the increase in strand growth resulting from dilution of the soil with sand; this increase is highly significant. The other noteworthy feature is the retarded growth of strands in the acid-treated whole soil at 40% saturation; this retardation may be ascribed to the joint action of three adverse factors, viz. acidity, low soil moisture, and a less than optimum soil texture. The aggregate effect of these three adverse factors acting together seems to be greater than the sum of their effects acting singly, as seen in other combinations of soil reaction, moisture content and texture. Thus under the favourable conditions of the soil/sand mixture acidity did not depress growth of strands at all (owing to the greater 'availability' of water in soil/sand mixture, moisture content would not be expected to limit strand growth at 40% saturation). Again, in the whole soils, a decrease in soil moisture from 60 to 40% saturation appreciably depressed growth only in the acid-treated soil, and not in the untreated or limed soils. These results recall those of Brown (1922), who found that toxic concentrations of carbon dioxide depressed the growth of fungi in culture most greatly when other conditions were also adverse, e.g. under conditions of low temperature and low concentrations of nutrients. Brown's general conclusion that 'the retarding factor has greatest effect when the energy of growth is small' helps to clarify, if not to explain, the results of the above experiment with *Helicobasidium purpureum.*

Experiments with Rossi-Cholodny slides

The Rossi-Cholodny glass slides used for this and the following experiments measured approximately 8·2 × 5·4 cm., and were made by cutting old lantern slide glasses in half. In setting up the slides, part of the dry soil was first added to each jar and the slides fixed in their vertical position

before the soil was moistened. Inoculum disks were then placed, mycelial surface against the glass slides, in position just touching the surface of the moistened soil, and the remainder of the dry soil was then added, shaken down, and brought to the correct moisture content.

Experiment VIII. This first experiment was designed to compare growth of *Helicobasidium purpureum* along the slides with that over potato tubers, and was carried out in allotment soil, at a moisture content of 60% saturation. The inoculum disks were taken from the same plates, one day older, as those for Experiment VII. Ten slides and twenty tubers, variety Majestic, were inoculated; measurements of mycelial growth over the slides were made after three weeks, and of strand growth over the tubers after three and again after five and a half weeks (mean laboratory temperature 17° C.). Results are given in Table 7.

Table 7. *Maximum growth of mycelium in mm.*

	Slides	Tubers
After 3 weeks	27	11
After 5½ weeks	—	30

In the first three weeks, *Helicobasidium purpureum* grew more than twice as far over the slides as over the tubers. Although it is easier to detect mycelium on the slides than the apical portions of strands on the tubers, yet appreciable underestimate of strand growth is considered unlikely, because in the second period of two and a half weeks, strand growth over the tubers was twice as fast (by extrapolation, 23 mm. in three weeks as against 27 mm in three weeks over the slides). The apparently slower growth of strands over the tubers than of mycelium over the slides is therefore due chiefly to an initial check after inoculation of the tubers.

Experiment IX. Growth over the slides was compared in three lots of allotment soil, previously treated with 1% sulphuric acid, untreated, and previously treated with 0·25% calcium hydroxide, at three different moisture contents, 40, 60 and 80% saturation. Submarginal inoculum disks were taken from colonies aged three and a half weeks at 25° C., on plates poured at normal depth with 2 meat-2 malt agar. Maximum growth of mycelium over the slides after twenty-four days (mean laboratory temperature 16° C.) is given in Table 8.

Table 8. *Maximum growth of mycelium in mm.*

	Acid-treated soil	Untreated soil	Limed soil
40% saturation	16	26	24
60% saturation	17	26	27
80% saturation	21	31	25
	*p*H values of soil		
Start of exp.	5·9	7·3	8·0
End of exp.	6·6	7·4	7·8

The depressing effect of soil acidity on growth of mycelium over the slides, to be observed in Table 8, was confirmed in a further experiment, in

which twenty slides were buried in acid-treated soil (1·25% sulphuric acid: *p*H 5·6) and twenty in untreated soil (*p*H 7·3) at a moisture content of 60% saturation. Growth of mycelium over the slides after twenty-four days (mean laboratory temperature 16° C.) was 19 ± 1·7 mm. in the acid-treated and 29 ± 0·9 mm. in the untreated soil.

Summary

For this investigation, potato tubers were inoculated with 8·5 mm. disks cut from a growing colony of *Helicobasidium purpureum* on meat-malt extract agar, and buried in glass jars filled with soil; maximum growth of mycelial strands from the inoculum disk was measured after some seven weeks by a paper scale fitted to the curved surface of the tuber.

The distance covered by mycelial strands and the frequency of infection cushions decreased with increase in storage age of the tuber. Inoculum disks cut from the growing margin of the fungus colony (marginal disks) were less effective for production and growth of strands than disks cut within a circle drawn 8·5 mm. from the margin (submarginal disks). The greater effectiveness of submarginal disks was related to their greater mycelial density; progressive development of mycelium within the marginal zone of the fungus colony was due to infiltration of new hyphae from the submarginal zone, as well as to branching of the original hyphae within the marginal zone. Production and growth of strands also increased with concentration of nutrients in the agar, and especially with that of the malt constituent. With increase in age of the colony to five or six weeks at 25° C., the potency of inoculum disks for strand production declined, but this apparent staling effect did not occur with disks cut from colonies on double depth agar (6 mm.). Transfer of colonies after three or four weeks' growth at 25° C. to laboratory temperature (15–20° C.) for a further 1–2 weeks reduced strand growth from disks more severely than did continued incubation of the colonies at 25° C. for the same period.

Growth of strands was greater in a 1 soil : 3 sand mixture than in soil. Soil acidity depressed strand growth at medium and low but not at high soil moisture content, and not in the soil/sand mixture. This adverse effect of soil acidity was also shown in growth of mycelium over Rossi-Cholodny slides.

I am indebted to Mr W. Buddin and Dr R. Hull for valued assistance, and especially for enabling me to see occurrences of violet root rot.

REFERENCES

Blair, I. D. (1943). Behaviour of the fungus *Rhizoctonia Solani* Kühn in the soil. *Ann. appl. Biol.* xxx, 118–27.

British Mycological Society (1944). *List of Common British Plant Diseases*, 3rd ed.

Brown, W. (1922). On the germination and growth of fungi at various temperatures and in various concentrations of oxygen and of carbon dioxide. *Ann. Bot., Lond.*, xxxvi, 257–83.

Brown, W. (1923). Experiments on the growth of fungi on culture media. *Ann. Bot., Lond.*, xxxvii, 105–29.

Fig. 1

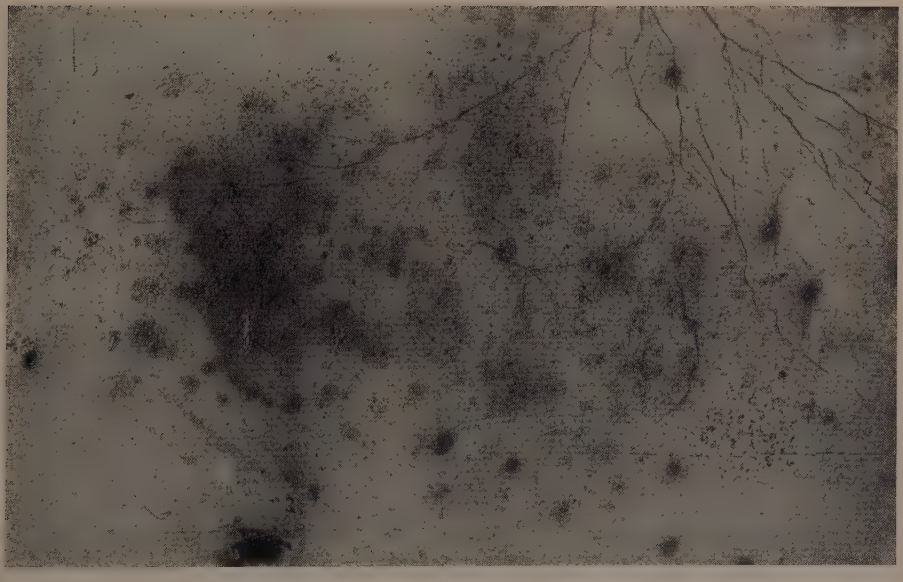


Fig. 2

Fig. 1

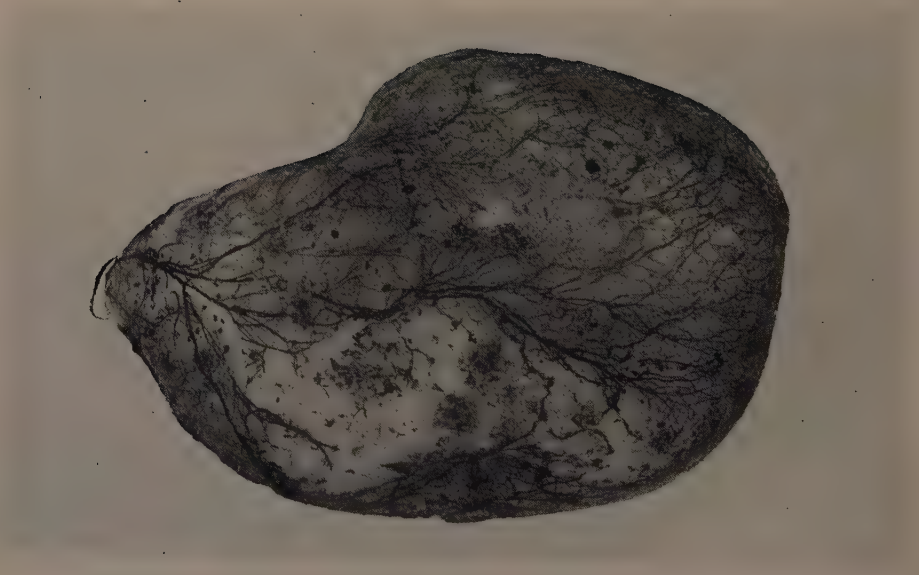


Fig. 2

Buddin, W. & Wakefield, E. M. (1924). Some observations on the growth of *Rhizoctonia Crocorum* (Pers.) DC. in pure culture. *Ann. appl. Biol.* xi, 292–309.
Buddin, W. & Wakefield, E. M.(1927). Studies on *Rhizoctonia Crocorum* (Pers.) DC. and *Helicobasidium purpureum* (Tul.) Pat. *Trans. Brit. myc. Soc.* xii, 116–40.
Buddin, W. & Wakefield, E. M. (1929). Further notes on the connexion between *Rhizoctonia Crocorum* and *Helicobasidium purpureum*. *Trans. Brit. myc. Soc.* xiv, 97–9.
Candolle, A. P. de (1815). Mémoire sur les rhizoctones, nouveau genre de champignons qui attaque les racines des plantes et en particulier celle de la luzerne cultivée. *Mém. Mus. Hist. nat.* ii, 209–16.
Duggar, B. M. (1915). *Rhizoctonia Crocorum* (Pers.) DC. and *R. Solani* Kühn (*Corticium vagum* B. & C.) with notes on other species. *Ann. Mo. bot. Gdn*, ii, 403–58.
Duhamel, Monceau (1728). Explication physique d'une maladie qui fait périr plusieurs plantes. *Hist. Acad. Roy. Sci. Paris*, pp. 100–12.
Faris, J. A. (1921). Violet root rot (*Rhizoctonia Crocorum* DC.) in the United States. *Phytopathology*, xi, 412–23.
Fries, E. M. (1823). *Systema Mycologicum*, ii, 265–6.
Garrett, S. D. (1936). Soil conditions and the take-all disease of wheat. *Ann. appl. Biol.* xxiii, 667–99.
Hull, R. & Wilson, A. R. (1946). Violet root rot of sugar beet caused by *Helicobasidium purpureum* Pat. *Ann. appl. Biol.* (in the press).
Keen, B. A. & Raczkowski, H. (1921). The relation between the clay content and certain physical properties of a soil. *J. agric. Sci.* xi, 441–9.
Moore, W. C. (1943). Diseases of crop plants. A ten years' review (1933–42). *Bull. Minist. Agric. Fish. Lond.*, no. 126.
Persoon, C. H. (1801). *Synopsis Methodica Fungorum.*
Prillieux, E. (1891). Sur la pénétration de la Rhizoctone violette dans les racines de la betterave and de la luzerne. *C.R. Acad. Sci., Paris*, cxiii, 1072–4.
Russell, E. W. (1938). Soil structure. *Tech. Commun. Bur. Soil Sci., Harpenden*, no. 37.
Samuel, G. & Garrett, S. D. (1945). The infected root hair count for estimating the activity of *Plasmodiophora Brassicae* Woron. in the soil. *Ann. appl. Biol.* xxxii, 96–101.
Shen, C. I. (1940). Soil conditions and the *Fusarium culmorum* seedling blight of wheat. *Ann. appl. Biol.* xxvii, 323–9.
Tulasne, L. & C. (1851). *Fungi Hypogaei.*

EXPLANATION OF PLATES

Plate V

Fig. 1. Potato tuber inoculated with agar disk of *Helicobasidium purpureum*, showing growth of mycelial strands.
Fig. 2. Surface of tuber inoculated with *H. purpureum*, showing mycelial strands and infection cushions (visible as dark points distributed over areas of local discoloration).

Plate VI

Fig. 1. Progressive infection and rotting by *H. purpureum*, in two potato tubers collected in field by Mr W. Buddin.
Fig. 2. Epiphytic strands of *H. purpureum* on surface of potato tuber collected in field by Mr W. Buddin.

(*Accepted for publication* 5 *April* 1946)

STUDIES ON BRITISH CHYTRIDS

I. *DANGEARDIA MAMMILLATA* SCHRÖDER

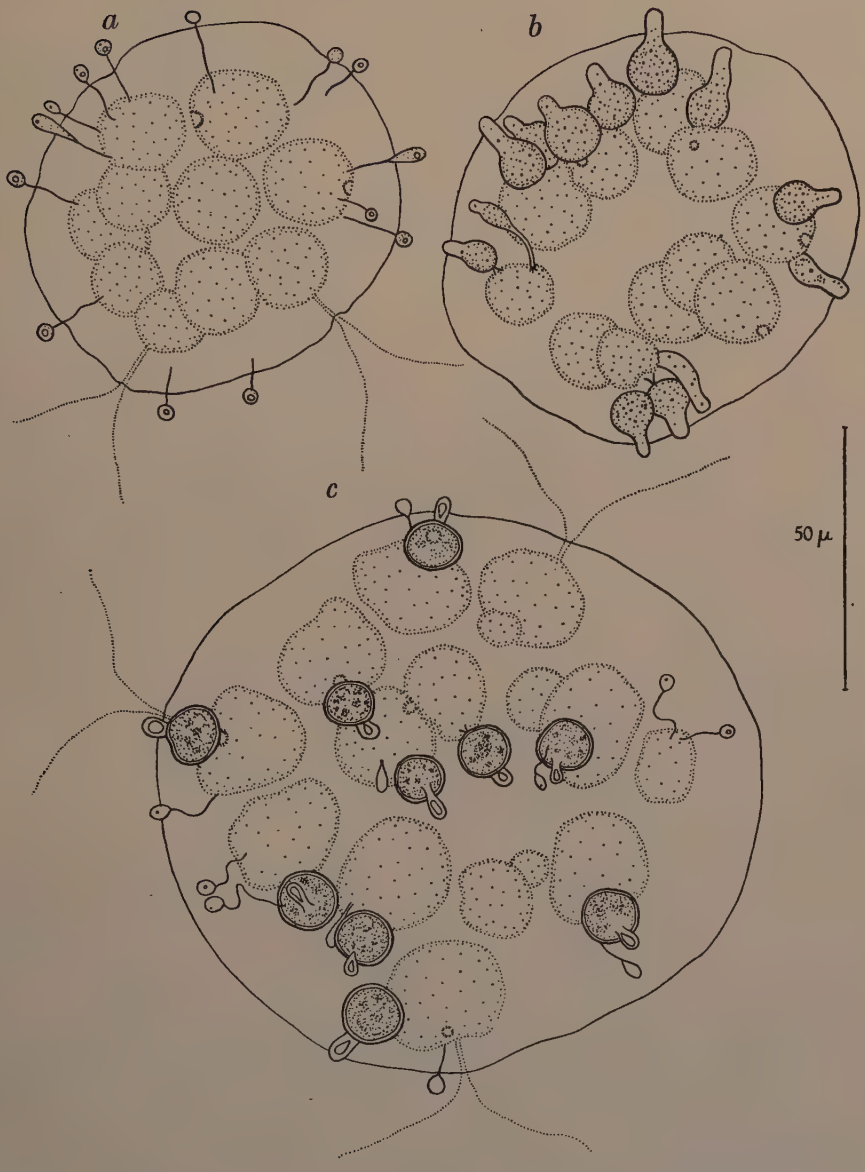
By HILDA M. CANTER, B.Sc.
Department of Botany, Queen Mary College, London

(With Plate VII and 5 Text-figures)

In the plankton of Barn Elms Reservoir (No. 5), Hammersmith, London, *Eudorina elegans* Ehrenb. was found from November 1945 to January 1946 to be infected by a chytridiaceous fungus bearing a striking resemblance to *Dangeardia mammillata* which Schröder (1898) originally described on *Pandorina morum* Bory. A detailed study was made of the life history of this fungus based entirely on observations from living material.

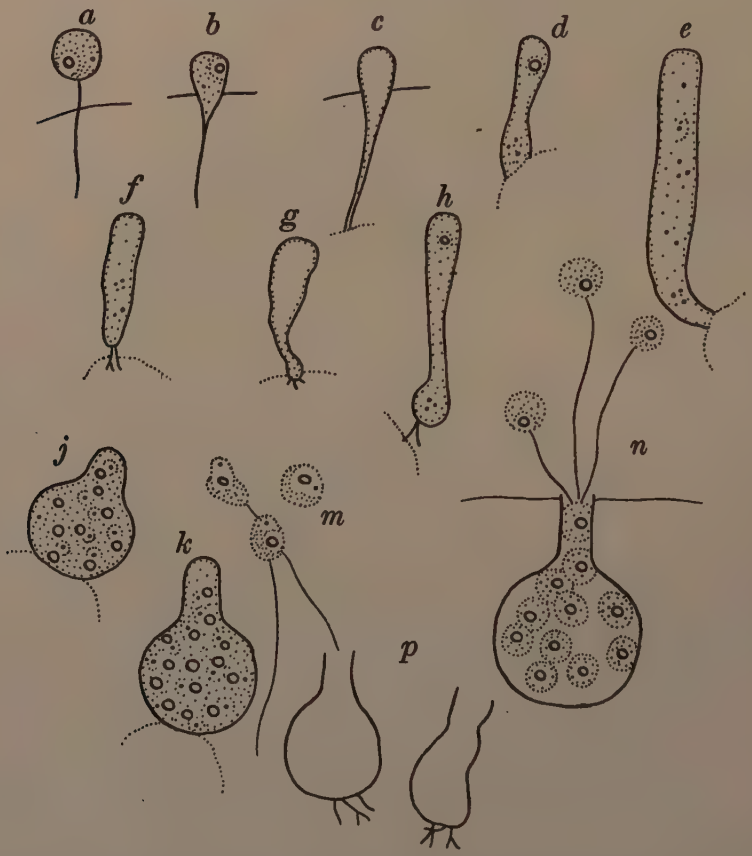
In the parasitized coenobium the chloroplast of each infected cell contracts away from the wall, and is finally reduced to a small mass of yellowish brown granules. As many as thirty individuals of the parasite are sometimes to be seen in a single coenobium, but, provided that one or two cells remain unaffected, the host retains its motility.

The story of development of the parasite has been built up by the examination of a large number of individuals in varying stages. The zoospore encysts on the surface of the coenobium and produces a fine germ tube which grows through the mucilage sheath to the nearest host cell (Text-fig. 1*a*). After contact is made with a host cell the germ tube gradually broadens from the proximal towards the distal end, until the thallus is almost cylindrical. The base then continues to swell, and the developing sporangium becomes flask-shaped, embedded, except for the extreme apex of the neck, in the gelatinous sheath of the *Eudorina* coenobium (Pl. VII, fig. 1). The mature sporangia range in size from 7 to 15 μ in diameter by 10 to 24 μ in length. In several examples, before the germ tube had become equally broad along all its length, the basal swelling had already begun to develop and bore a few rhizoids. The rhizoids are numerous, short, usually unbranched structures (3 μ in length) arising in a tuft from the base of the sporangium and penetrating the host cell (Text-fig. 2*f*, *g*, *h*, *p*). The changes in the protoplasm during spore development are similar to those described for most chytrids, and at maturity the sporangium contains a number of similar, and equally spaced, refractive globules, each indicating the position of a zoospore. A mature sporangium may produce from twenty to one hundred zoospores which emerge singly, squeezing through the opening formed upon deliquescence of the apex (Text-fig. 2*n*). Immediately the first zoospores have emerged the remainder begin to move over one another, and a swarming mass of them is to be seen in the sporangium which empties, however, within 5–10 min. after dehiscence. The empty sporangium does not collapse immediately, but fairly soon shows signs of shrivelling.



Text-fig. 1. *Dangeardia mammillata*. *a*, young thalli each consisting of an encysted zoospore and thread-like germ tube. *b*, a number of nearly mature sporangia. *c*, resting spores, some with associated male thalli. All × 700.

The zoospores appear to be of two types, and each sporangium produces one kind only. The sporangia themselves are morphologically indistinguishable. Some zoospores are spherical, 2·5 μ in diameter with a single conspicuous oil globule surrounded by an area of less dense protoplasm, and have a single posterior flagellum 15 μ long. Others have, in addition to the oil globule, a minute rod-shaped oscillating granule in an anterior

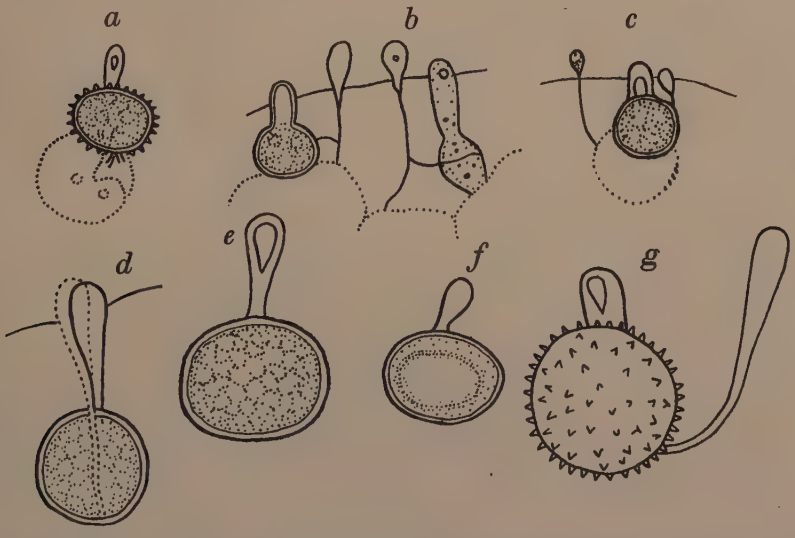


Text-fig. 2. *Dangeardia mammillata*. *a–h*, stages in early development of thallus. *j*, *k*, mature sporangia, each oil globule indicates the position of a zoospore. *m*, zoospores with oscillating granule in addition to the oil globule. *n*, zoospores with oil globule but no granule, escaping from sporangium. *p*, two empty sporangia showing rhizoids. *d*, × 1333; the rest × 1777.

position (Text-fig. 2*m*). These zoospores are often somewhat oval (3·5 × 1·8 μ), and appear to be slightly flattened.

In collections made during November zoosporangia only were observed, but by mid-December sexually formed resting spores had appeared (Text-fig. 3, and Pl. VII, figs. 2–4). In resting-spore formation, union occurs between a relatively large flask-shaped thallus (presumably the female), almost identical with a zoosporangium, and a relatively small one (pre-

sumably the male), resembling an early stage in the development of an asexual sporangium. There were certain strong indications that the male thalli were derived only from zoospores of the type with a moving granule, but further work in single-spore cultures seems necessary before such a remarkable state of affairs can be established.

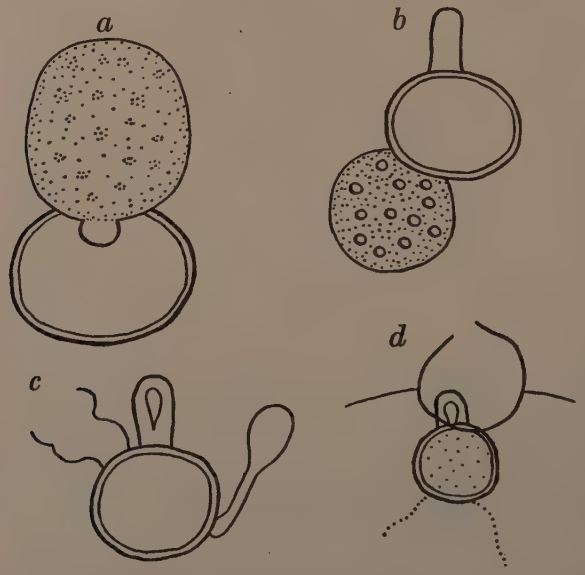


Text-fig. 3. *Dangeardia mammillata*. *a*, spiny resting spore with rhizoids, × 933. *b*, two young resting spores with associated male thalli, having branched germ tubes, × 1333. *c*, smooth-walled resting spore with two males; the one on the right still retains some of its contents, × 933. *d*, *e*, resting spores showing clavate appendage, derived from the zoospore and its germ tube, × 1777; in *d*, the male thallus is drawn with a broken outline. *f*, resting spore with a single large oil globule, × 1300. *g*, spiny-walled spore with male thallus, × 1777. In figs. *d*, *f*, the appendage is completely filled with refractive material; in figs. *a*, *c*, *e* and *g*, a centrally unthickened area remains.

Text-fig. 4. Stages in the development of a resting spore of *Dangeardia mammillata*, according to Schröder (1898).

The young resting spore can be recognized not only by the presence of an associated male thallus (Pl. VII, fig. 4), but also by the clavate germ tube and by the appearance of the oily contents. The germ tube of the male thallus makes direct contact with the swollen base of the female, and the empty

male normally connected with each mature resting spore indicates that the contents of the male pass into the female. Further evidence was afforded by the fact that where two males were associated with a female thallus one was empty, whereas the other still retained its contents. The germ tube of the male thallus was very rarely seen to branch (Text-fig. 3*b*). While a male thallus was found connected with most of the resting spores, it appeared to be absent from a few, and these spores may have developed parthenogenetically. As development proceeds, presumably following fusion, the wall of the neck of the female thallus begins to thicken from the outside towards the centre. A solid plug of highly refractive material is normally produced, but in some specimens, which were perhaps immature,



Text-fig. 5. *Dangeardia mammillata*. *a*, germinated resting spore with immature germ-sporangium on its surface, × 1777. *b*, germinated resting spore with almost mature germ-sporangium, each oil globule indicates the position of a zoospore, × 1777. *c*, germinated resting spore with associated male thallus and empty shrivelled wall of the germ-sporangium, × 1777. *d*, empty resting spore with granulated wall, the granules on the surface of the wall are visible after germination, × 1300.

a centrally unthickened area remained (Text-fig. 3*e*, *g*). The length of this neck varies, and it is often characteristically narrowed towards its attachment to the swollen base of the female thallus (Pl. VII, fig. 3), which forms the actual resting spore. The latter at maturity is oval to spherical in shape, 7·5–13 μ in diameter, and has a thick wall which may be smooth, granular or distinctly spiny. The contents are usually oily with numerous scattered droplets, but several specimens were seen with a single large oil globule (Text-fig. 3*f*). The zygote germinates readily under laboratory conditions. A thin-walled sporangium is formed on the surface of the resting spore, which is left without protoplasmic contents, thus behaving as a prosporangium in germination (Text-fig. 5*a*, *b*). Actual dehiscence of these

sporangia was not seen, but from observations on mature and dehisced specimens from which the zoospores were still escaping, it is clear that posteriorly uniflagellate zoospores are produced. It also seems that as in the zoosporangia, the germ-sporangia produce zoospores all of one type, either with a single oil globule or with an additional oscillating granule. Zoospores with an oscillating granule are not unknown among chytrids. Hanson (1944) describes such zoospores as typical of *Loborhiza Metzneri*, a parasite on *Volvox*.

The type of sexuality exhibited by *Dangeardia* most nearly resembles that described for *Zygorhizidium Willei* Löwenthal. In the latter the male thallus similarly produces a conjugation tube which makes direct contact with a swollen female thallus.

Since *Dangeardia mammillata* was originally described by Schröder (1898) few references to it have appeared in the literature. Skvortzow (1927) gives a brief description of a fungus from Manchuria which he identified with *Dangeardia*, but since he observed neither the rhizoids nor the resting spores, and the zoospores were more than twice the diameter of those described by Schröder, the identity of this chytrid remains uncertain. Ingold (1940) found what he believes to be the same organism parasitizing *Eudorina elegans* in the plankton of Swithland Reservoir, Leicestershire, England. He did not, however, observe the resting spores (private communication). Sparrow (1943) recorded it from America as a citation from a personal communication with Bartsch. Bartsch (*in lit.*) indicated that his observations agreed with those of Schröder.

Although the chytrid described in this paper agrees well with *Dangeardia mammillata*, it nevertheless departs from the original description in one major aspect, namely, that the resting spores are epibiotic and sexually formed. That Schröder (see Text-fig. 4) may have interpreted an epibiotic resting spore as endobiotic is not unlikely, for a spore, when viewed immediately above a host cell (especially if the chloroplast is rather shrunken), often gives the appearance, at first sight, of being endobiotic. Other characteristics of the resting spore as described by Schröder were the clavate appendage (derived from the zoospore and its germ tube), the spiny wall, and single oil globule. All these features have been observed in the material examined by me (see Text-fig. 3, and Pl. VII, figs. 2, 3), leaving little doubt as to the identity of the fungus.

Hood (1910) described *Rhizophydium Eudorinae*, a parasite on *Eudorina elegans*, which in its method of development, shape, size and inoperculate nature of the sporangia, and size of the zoospores agrees with *Dangeardia*. However, she saw no rhizoids and the zoospores were described as emerging in a mass embedded in mucilage. The spherical resting spore which Hood associated with *Rhizophydium Eudorinae* is unlike that of *Dangeardia*, but may possibly belong to another fungus. Her figures of *Rhizophydium Eudorinae* suggest that at least two chytrids were present on the *Eudorina*. An epibiotic species is shown in her paper (1910, Text-fig. 5) under abnormalities of *Rhizophydium Eudorinae*.

Dangeardia mammillata seems to differ sufficiently from other chytrids to justify its retention in a separate genus, but an alteration in the definition

of the genus is necessary, since the resting spore is epibiotic, and not endobiotic as originally described. It is further suggested that *Rhizophydium Eudorinae* is not a valid species, but would seem to represent material of *Dangeardia mammillata* together with an epibiotic chytrid belonging possibly to *Rhizophydium* or *Chytridium*.

My thanks are due to the Metropolitan Water Board for permission to collect samples of plankton from Barn Elms Reservoir, and especially to Prof. C. T. Ingold for the helpful advice and criticism he has given throughout the course of this work.

REFERENCES

Hanson, A. (1944). A new chytrid parasitizing *Volvox*; *Loborhiza Metzneri* gen.nov., sp.nov. *Amer. J. Bot.* xxxi, 166–71.

Hood, O. (1910). On *Rhizophydium Eudorinae*, a new chytridiaceous fungus. *Proc. Birmingham Nat. Hist. Phil. Soc.* xii, 38–45.

Ingold, C. T. (1940). *Endocoenobium Eudorinae*, gen. et sp.nov., a chytridiaceous fungus parasitizing *Eudorina elegans* Ehrenb. *New Phytol.* xxxix, 97–103.

Schröder, B. (1898). *Dangeardia*, ein neues Chytridineen Genus auf *Pandorina morum* Bory. *Ber. dtsch. bot. Ges.* xvi, 314–21.

Skvortzow, B. W. (1927). Über einige Phycomyceten aus China. *Arch. Protistenk.* lvii, 204–6.

Sparrow, F. K. (1943). *Aquatic Phycomycetes*. Ann Arbor, U.S.A.: University of Michigan Press.

EXPLANATION OF PLATE VII

Dangeardia mammillata Schröder

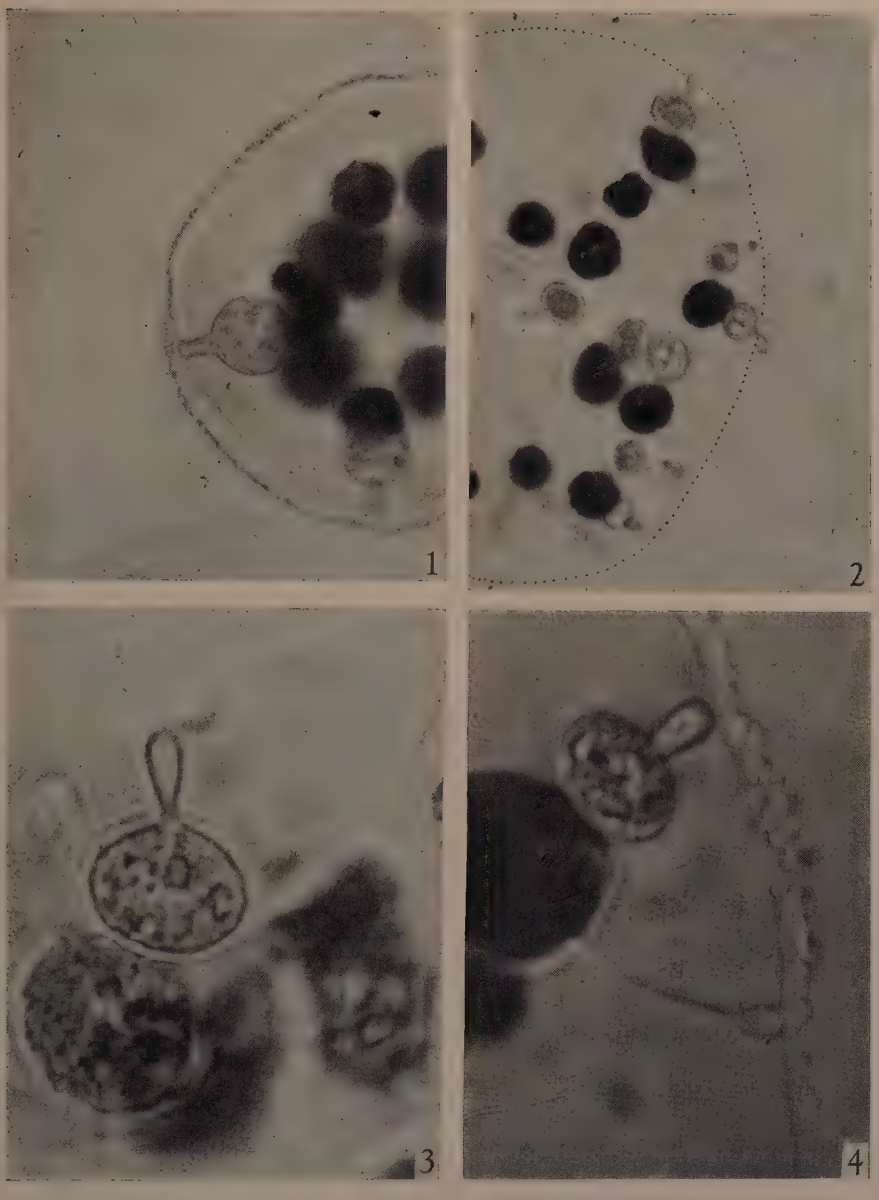
Fig. 1. Part of a *Eudorina* colony showing two sporangia. × 1300.

Fig. 2. Part of a *Eudorina* colony with resting spores; one near the centre shows a single large oil globule. The mucilage sheath of the host is indicated by a dotted line. × 900.

Fig. 3. Resting spore with granular wall and clavate appendage filled with refractive material. × 2500.

Fig. 4. Above resting spore, below empty male thallus; contact between them is obscured by the dense contents of the host chloroplast. × 2666.

(*Accepted for publication* 17 *April* 1946)


1
2
3
4

NEW CHYTRIDIACEOUS FUNGI FROM CAMBRIDGE

By R. H. HASKINS

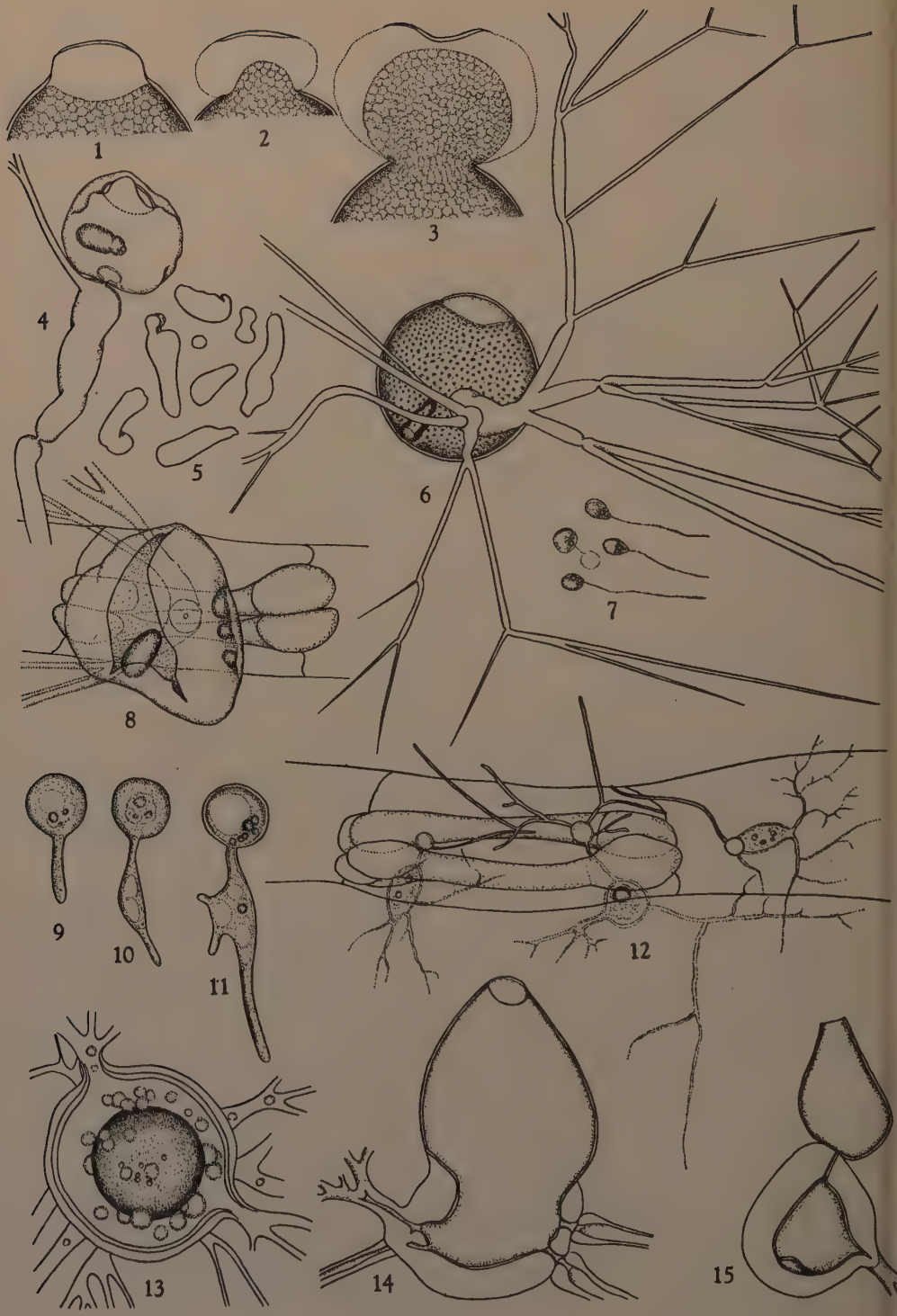
(With Plate VIII and 15 Text-figures)

In July 1945, collections of saprophytic chytrids were made in Cambridge, England, and its vicinity. Boiled hempseed, dechlorophyllized grass, maize and wheat leaves, filter paper and regenerated cellulose film, serving as baits, were placed in various ditches and ponds. For convenience in retrieving the baits, they were placed in muslin or gauze bags and held under water in small wire cages. After from one to three weeks, the baits were brought into the laboratory and examined. In some pieces, the presence of saprophytic chytrids could be detected at once. All baits were carefully washed in a gentle stream of tap water, and placed in sterile Petri dishes containing sterile charcoal water (distilled water filtered through animal charcoal, then autoclaved), and fresh pieces of bait added.

These gross cultures were examined from time to time and a number of species of chytrids and other aquatic fungi were identified. Many of the fungi identified have already been reported for the area by Sparrow (1936) and therefore will not be mentioned here. Species of the following genera were set aside for future study: *Olpidium* (in rotifers), *Olpidiopsis* (in *Achlya* and *Saprolegnia*), *Lagenidium* (in desmids), *Rhizidiomyces* (on insect exuviae and similar to *R. Hansonii* Karling (Karling, 1944)), *Diplophlyctis* (on grass and regenerated cellulose film), *Cladochytrium* (in grass, *C. hyalinum* Berdan (Berdan, 1941)), and *Nowakowskiella* (on grass and regenerated cellulose film, *N. hemisphaerospora* Shanor). Four of the forms collected, however, were given special attention, and two are briefly described in this preliminary paper.

Using methods and technique described by Berdan (1939) and Haskins (1939*a*), the new forms were brought into unifungal culture. Unifungal cultures are satisfactory for morphological descriptions, and the writer therefore made no attempt to procure 'pure' cultures. Such work is now in progress following the methods of Stanier (1942). The cultures from which these studies were made were unisporangial.

One of the forms (Text-figs. 1–8) was at first wrongly identified as *Rhizidium ramosum* Sparrow. Closer examination proved the sporangia to be operculate (Text-figs. 1–3). It cannot be included in the operculate genus *Chytridium* because its zoosporangia do not develop endo-exogenously (see Karling, 1936), but develop directly from the body of the encysted zoospore. It was therefore considered advisable to erect a new genus to include monocentric, operculate, rhizidiaceous chytrids in which the zoosporangia arise from the enlargement of the encysted zoospore and in which the resting sporangium is epibiotic or extramatrical. A paper was prepared describing and naming the genus and the type species. When the



Text-figs. 1–15

description of the new genus *Chytriomyces* (Karling, 1945) was received from America towards the end of August, it was immediately obvious that the genera were identical, and a revision of the present paper was undertaken. The fungus described in this paper is sufficiently different from the two species for which Karling erected the genus to be considered new. The species is characterized by variously shaped, refractive nodules (Text-figs. 4–6, 8) that are borne on the interior surface of the zoosporangial wall, and is accordingly named *Chytriomyces nodulatus*.

Chytriomyces nodulatus sp.nov. Zoosporangio hyalino, sphaerico, 15–50 μ dia. (plerumque 33 μ), cum tumoribus 1–20 nodosis, hyalinis, refractivis, variatim formatis, a pariete sporangiali alioqui levissimo ad centrum eminentibus, exteriora simul parietis nonnunquam irregularia efficientibus; interioribus granulatis, globulis aeque plerumque distributis, spatio sub ipso operculo vacuo. Operculo apiculato–subapiculato, brevi, neque in vacuo sporangio permanente; orificio circulari 12–18 μ dia., pariete sporangiali saepe post dehiscentiam scisso. Subsporangiali tumore variatim formato vel absente, ad 10 μ dia., a maturo sporangio pariete quodam intercepto. Rhizoideo systemate (addito subsporangiali tumore) 30–300 μ magnitudine. Rhizoidibus tenuissimis, 0·5–5·0 μ dia., ramis dichotomis, nonnunquam propter obstantia constrictis. Zoosporis partim adultis primum eiectis, deinde in vesicula evanescenti compressis; hyalinis, ovatis–pyriformibus, 3·0–4·5 μ, uninucleatis, cum uno (rarissime compluribus) globulo refractivo 1·1 μ dia. prope nexum flagelli; uniflagellatis; flagello sex vel octo partibus zoosporo longitudine maiore, cauda praedito. Infuso ZnClI interiora parietis zoosporangialis paululum temporis suppupurea manent; spatium illud sub rubro operculo vacuum neutrali intra-vitam rubescit rubore, donec dehiscit. Sporangiis perdurantibus suffuscis, levibus, sphaericis, 10–35 μ dia., cum uno vel compluribus magnis, minoribus parietalibus globulis, exstantibus prosporangiis ad germinationem.

Chytriomyces nodulatus n.sp. Zoosporangia hyaline, spherical, 15–50 μ in diameter (average 33 μ), with one to twenty hyaline, refractive, variously shaped, nodular protuberances extending centripetally from the otherwise smooth sporangial wall, and sometimes causing irregularities on the outer surface; contents granular with more or less evenly distributed globules; area immediately under the operculum clear. Operculum apical to subapical, shallow, not persistent on the empty sporangium; orifice circular,

---

Legends to Text-figs. 1–15

Text-figs. 1–8. *Chytriomyces nodulatus*. Figs. 1–3. Stages of dehiscence of zoosporangium showing operculum and vesicle. × 924, 693, 785 respectively. Fig. 4. Emptied zoosporangium with nodule. Note invaginated cross-wall separating zoosporangium from rhizoidal system. × 693. Fig. 5. Nodules from various sporangia. × 739. Fig. 6. Mature thallus. × 485. Fig. 7. Zoospores. × 785. Fig. 8. Emptied sporangium over stoma of substratum. Note splitting of zoosporangial wall, nodule, and dichotomy of subsporangial swelling. × 693.

Text-figs. 9–15. *Entophlyctis aurea*. Figs. 9–11. Three successive stages in germination of zoospore. × 1663. Fig. 12. Young stages of thallus development showing infection through stomatal pore and directly through substratum wall. × 462. Fig. 13. Resting sporangium. × 601. Figs. 14, 15. Germinating resting sporangia. Drawings made with camera lucida. × 739.

12–18 μ in diameter, sporangial wall often splitting after dehiscence. Subsporangial swelling variously shaped or absent, diameter up to 10 μ, cut off by a cross-wall from the mature sporangium. Rhizoidal system including subsporangial swelling 30–300 μ in extent. Rhizoids delicate, from 0·5–5·0 μ in diameter, branching dichotomous with constrictions where passing through obstructions. Zoospores, partially developed, discharged into, and later swarming in, an evanescent vesicle; hyaline, oval to pyriform, 3·0–4·5 μ, uninucleate with single (rarely several) refractive globule 1·1 μ in diameter near point of attachment of flagellum; uniflagellate, flagellum six to eight times diameter of zoospore in length, with tail-piece. Treatment with chloriodide of zinc producing short-lasting, extremely faint mauve coloration of inner surface of the zoosporangial wall; with intra-vitam neutral red operculum staining red, clear zone beneath operculum reddening, until dehiscence. Resting sporangia brownish yellow, smooth, spherical, 10–35 μ in diameter with one or several large globules and parietal smaller ones; functioning as prosporangia in germination.

Saprophytic in leaves of wheat, maize, oats and various grasses, and in insect exuviae, submerged in water, Cambridge, England.

The second of the forms (Text-figs. 9–15 and Pl. VIII) was the large, monocentric, orange-coloured, rhizidiaceous chytrid previously described as *Rhizophlyctis* (?) *Petersenii* Sparrow by Haskins (1939*a*, 1939*b*). Careful investigation of the early stages of development showed that the sporangium develops from a swelling on the germination tube and not directly from the body of the encysted zoospore. Attempts to grow this form on insect exuviae on which Sparrow (1937) found *R. Petersenii* were unsuccessful. This chytrid is now placed in its correct genus *Entophlyctis*, and because of the distinct bright orange colour of the mature sporangium is named *Entophlyctis aurea*.

Entophlyctis aurea sp.nov. Zoosporangiis plerumque intramatricalibus, sphaericis, vel variatim formatis, 15–470 μ dia.; interioribus luteis–subluteis iuvenilibus, croceis maturis; pariete hyalino et stratificato, strati intimi reactione cellulosa (ZnClI) conspicua; strato extremo haud fucato. Tubulis exeuntibus 1–10, curtis, marginibus reflexis, 4·2–21 μ dia. ad orificium, 5·3–35·7 μ ad basin, 6·3–50·3 μ altitudine, gelatina clausis. Rhizoidibus locis 1–20 e sporangio eminentibus, robustis, ad 63 μ dia. ad originem, a maturo sporangio parietibus cellulosis interceptis, ramosissimis, ad 750 μ long. Zoosporis sphaericis–subsphaericis, 4·5–5·2 μ dia., cum compluribus granulis vel globulis parvis refractivisque; uniflagellatis; flagello quinque partibus zoosporo maiore longitudine; zoosporis permultis, emergentibus initio per unum tubulum exeuntem in massam sphaericam vel irregularem, primum quiescentibus, deinde enatantibus, reliquis zoosporis per cunctos effugientibus tubulos, labentibus vel emicantibus, interdum amoebaeis. Sporis perdurantibus phora formaque zoosporangiis similibus, 22–106 μ dia., interioribus cum singulis vel compluribus croceo-fuscis globulis (11·4–60·8 μ dia.), fortasse cum minoribus in peripheria globulis sine colore; pariete crasso (3–10 μ), hyalino, stratificato, strati interioris reactione cellulosa in germinatione conspicua;

prosporangii vice fungentibus in germinatione ut surgat per porum exiguum zoosporangium extramatricale evanescens, vel per hiatum latissimum zoosporangium sacculo simile.

Entophlyctis aurea n.sp. Zoosporangia usually intramatrical, spherical or variously shaped, 15–470 μ in diameter; contents yellow to pale orange in colour when young, bright orange at maturity; wall hyaline and layered, innermost layer giving marked cellulose reaction with chloriodide of zinc, outer layer not staining. Exit tubes one to ten, short with reflexed rims, 4·2–21 μ in diameter at orifice, 5·3–35·7 μ at base, 6·3–50·3 μ in height, with gelatinous plugs. Rhizoids arising from one to twenty places on the sporangium, stout, up to 63 μ in diameter at point of origin, cut off from mature sporangium by cellulose cross-walls; much branched, extending up to 750 μ in length. Zoospores spherical to subspherical, 4·5–5·2 μ in diameter, with several small refractive granules or globules; uniflagellate, flagellum five times diameter of zoospore in length; number of zoospores great, emerging initially through one exit tube to form a spherical or irregular mass, lying quiescent for a few moments before swimming away, remaining zoospores escaping through all exit tubes; movement gliding and darting, intermittently amoeboid. Resting spores borne and shaped similarly to zoosporangia, 22–106 μ in diameter, contents with one to several large orange-brown globules (11·4–60·8 μ in diameter), with or without smaller parietal colourless globules; wall thick (3–10 μ), hyaline, layered, inner layer at germination giving marked cellulose reaction; functioning as prosporangium in germination to give rise through a narrow pore to an extramatrical evanescent zoosporangium, or through a wide opening to a sac-like zoosporangium.

Saprophytic in various grasses, wheat, oat, and maize leaves, regenerated cellulose film, filter paper, and lens paper submerged in water, North Bay, Ontario, and London, Ontario, Canada (Haskins, 1939*a*), and Cambridge, England.

Summary

A new species, *Chytriomyces nodulatus*, is proposed for a chytrid of the genus *Chytriomyces*. This species is characterized by variously shaped, refractive nodules borne on the inner surface of the sporangial wall. It occurs saprophytically in leaves of grasses and cereals, vegetable debris, and insect exuviae submerged in water.

A new species, *Entophlyctis aurea*, is suggested for a large, monocentric, inoperculate, rhizidiaceous chytrid with bright orange contents in the mature sporangium. It is extremely variable in size and shape.

Both of these chytrids have been separated from other aquatic fungi found in baiting material placed in ponds and ditches near Cambridge, England, and have been subsequently cultured in England in unifungal state under as nearly sterile conditions as possible.

The author expresses his sincere thanks to Prof. F. T. Brooks, Botany School, Cambridge University, for placing at his disposal the laboratory and library facilities of the School, and for his many kindnesses and

encouraging interest; and to Dr J. D. Ralph, London, Canada, for the Latin diagnoses. The work was done while awaiting repatriation, on leave of absence from the Canadian Army Overseas. Author's present address: Department of Botany, University of Western Ontario, London, Ontario, Canada.

REFERENCES

Berdan, H. B. (1939). Two new genera of operculate chytrids. *Amer. J. Bot.* xxvi, 459–63.

Berdan, H. B. (1941). A developmental study of three saprophytic chytrids. I. *Cladochytrium hyalinum* sp.nov. *Amer. J. Bot.* xxviii, 422–36.

Haskins, R. H. (1939*a*). Cellulose as a substratum for saprophytic chytrids. *Amer. J. Bot.* xxvi, 635–9.

Haskins, R. H. (1939*b*). The life cycle of *Rhizophlyctis* (?) *Petersenii* Sparrow on cellophane as a substratum. Paper presented before Mycological Society of Amer., Columbus, Ohio, U.S.A.

Karling, J. S. (1936). The endo-exogenous method of growth and development of *Chytridium Lagenaria*. *Amer. J. Bot.* xxii, 439–52.

Karling, J. S. (1944). Brazilian Anisochytrids. *Amer. J. Bot.* xxxi, 391–7.

Karling, J. S. (1945). Brazilian chytrids. VI. *Rhopalophlyctis* and *Chytriomyces*, two new chitinophyllic, operculate genera. *Amer. J. Bot.* xxxii, 362–9.

Sparrow, F. K. Jr. (1936). A contribution to our knowledge of the aquatic Phycomycetes of Great Britain. *J. Linn. Soc. Lond.* (*Bot.*), l, 417–78.

Sparrow, F. K. Jr. (1937). Some chytridiaceous inhabitants of submerged insect exuviae. *Proc. Amer. Phil. Soc.* lxxviii, 25–53.

Stanier, R. Y. (1942). The cultivation and nutrient requirements of a chytridiaceous fungus, *Rhizophlyctis rosea*. *J. Bact.* xliii, 499–520.

EXPLANATION OF PLATE VIII

Figs. 16–21. *Entophlyctis aurea*. Fig. 16. Culture on regenerated cellulose film. Black dots are individual zoosporangia. From Kodachrome photomicrograph. ×0·76. Fig. 17. Mature spherical zoosporangium from culture on regenerated cellulose film. Note stout rhizoids and gelatinous globule at orifice of neck. ×204. Fig. 18. Mature zoosporangium from pure culture on agar showing surface exposed to air, and exit tube. ×239. Fig. 19. A mature and an empty zoosporangium from culture in corn leaf; stained with chloriodide of zinc. Inner wall shows marked cellulose reaction. ×239. Fig. 20. Resting sporangia in culture on regenerated cellulose film. ×111. Fig. 21. Germinating resting sporangium with large central globule still evident. ×299.

(*Accepted for publication* 2 *May* 1946)

NEW OR INTERESTING BRITISH FUNGI

By R. W. G. DENNIS and E. M. WAKEFIELD

(With 26 Text-figures)

The fungi here described have been accumulating over a period of years. To all who have assisted by sending in interesting material our thanks are due.

Anthurus Archeri (Berk.) Fischer in *Jahrb. k. bot. Gart. u. bot. Mus. Berlin*, IV, 81 (1886).

This beautiful phalloid, new to this country and no doubt an introduced alien, occurred in Trewidden Gardens, near Penzance, towards the end of October 1945. The finder, Pte N. E. M. Walters, R.A.P.C., had observed the 'eggs' among rotten stumps at the base of an *Escallonia*, and recognizing that it was something unusual sent material to Kew.

The genus *Anthurus* differs from *Lysurus* in that the arms of the receptacle are not sharply distinct from the stem, and are, at least at first, united with one another at the apices. In *Lysurus* the arms are distinct from the stem and usually quite free from one another at the tips.

Anthurus Archeri is described by Cunningham (1944) as follows: Unexpanded plant obovate, to 4 cm. diameter, usually smaller, exterior furfuraceous, dingy white. Receptacle with a short, stout, hollow, usually flaring stem, attenuate and white below, slightly expanded, open and red above, to 5 cm. long, often much less, 1–2·5 cm. diameter, divided directly into five to eight orange-red, simple arms, transversely rugulose on the interior, sutured longitudinally externally, chambered, apically united when freshly expanded, but often breaking away in older plants, varying from 3 to 7 cm. in length, occasionally bifurcate at the extremities. Spore mass borne on the inner surface of the arms, foetid, olivaceous, mucilaginous. Spores elliptical, 6–7·5 × 2–2·5 μ, epispore hyaline, smooth, 0·75 μ thick.

The Cornish specimen had seven arms, and two pairs at least were apically united. The spores measured 5–6 × 1·5–2 μ, a little smaller than the measurements given by Cunningham, but larger than those of most related phalloids. There seems little doubt that the plant can be referred to *A. Archeri*, a species widespread in Australia and Malaya.

Psilocybe cyanescens Wakefield, sp.nov.

Pileus 2–4 (rarius –7·5) cm. diam., e convexo expansus, glaber, hygrophanus, viscidus, udo castaneus, sicco flavidus vel ochraceus margine striatulo, tactu cyanescens: in statu juvenile cortina delicata nivea cum stipite conjunctus. Stipes albidus, sericeo-fibrillosus, 6–8 cm. longus, 2·5–5 mm. crassus, rarius ad 10 cm. × 7 mm., subrigidus, basi incrassatus et saepe incurvus, strigosus, tactu vel siccitate cyanescens. Lamellae e

cinnamomeo badio-fuscae, adnatae vel adnato-decurrentes, subdistantes, ad 5 mm. latae, acie pallidiore. Cheilocystidia lageniformia, circa 18–20 × 8 μ. Basidia tetraspora, obclavata, supra 8 μ crassa. Sporae sub lente flavo-brunneae, in massa fuscae, ellipticae, uno latere vix depressae, 10–12 × 6–7 × 5 μ, poro germinationis indistincto praeditae.

Hab. Ad terram, inter folia, ramenta, etc. in silvis, autumno, Hort. Bot. Reg. Kew.

This striking fungus has been known to the senior author for many years, and can usually be found in the autumn in the more woodland parts of Kew Gardens. It has, however, not been seen elsewhere, except once in the near neighbourhood of the Gardens, when it appeared to have originated from some rotting wood. The general appearance suggests a species near *Naucoria Myosotis*, but the spore-print is distinctly fuscous, and the decided indigo-blue colour which develops on both stem and pileus when handled distinguishes the fungus from anything which has been described in the genus *Naucoria*. A similar colour-change has been described in an American species of *Psilocybe*, *P. caerulescens* Murr., but that species is much more robust and the spores are smaller than those of the plant in question. The Kew plant in its micro- and macroscopic characters would probably be included in the genus *Deconica* W. G. Sm. by those who adopt this genus. It is retained in *Psilocybe* for the time being, until such time as a thorough revision of the purple-spored Agarics is forthcoming.*

Exobasidium Camelliae Shirai in *Bot. Mag., Tokio*, x, 51 (1896).

This fungus was described by Shirai (loc. cit.) as follows: 'Hymenium thick and white, forming a continuous layer all over the surface of the deformed organs, at first covered with a thick layer of subepidermal tissue, composed of 10 or more layers of cells, which it ruptures and breaks to a number of small pieces. Spores 4 to each basidium, oblong obovate, 14·5–17 × 7 μ. This species always attacks the flower buds of *Thea* (*Camellia*) *japonica* Nois, causing the hypertrophy and deformation of their parts. Very often the whole plant is reduced to an irregular mass of somewhat spherical form with a hollow interior, measuring 15 cm. or more in length. Common in Tokyo in May.' His Fig. 3 shows very long slender basidia with 2, 3, 3, 3, and 4 sterigmata respectively. Assuming a mean spore length of 15 μ the basidia drawn were about 100–120 μ long.

In June 1944 there was received at Kew an abnormal flower of *Camellia*, grown at Handcross, Sussex. Instead of the normal petals the whole flower had been transformed into a thick white, more or less globular structure. On close examination this gall was found to consist of a shallow cup with rounded and incurved edges, bearing on the outside some brown membranous fragments, apparently the remains of petals. The entire outer surface of the gall was covered with the powdery white hymenium of an *Exobasidium*, consisting of densely packed more or less cylindrical basidia gradually narrowing to their parent hyphae below. The exact base of such basidia is difficult to determine but their length appeared to be approximately 90–100 μ and their greatest width 7–9 μ. Each bore two very short

* See p. 165.

conical sterigmata. The spores were hyaline, elongated, usually more or less comma shaped, sometimes straight, widest at the apex and narrowed towards the curved base, 15–18(–22) × 5–7·5 μ, the majority about 18 × 7 μ. Mature spores were sometimes one- to three-septate.

This collection appears to differ from *E. Camelliae* in having only two sterigmata, in having the outer petals almost normal and in showing no evidence of a deep-seated origin of the hymenium. In other characters there appears to be considerable agreement with the diagnosis and it is proposed to refer the Sussex collection to that species meantime. *E. Euryae* Syd. & Bull., on inflorescences of *Eurya acuminata* in Nepal, differs in host genus and hymenial characters (basidia 60 × 5–10 μ, with 2–4 sterigmata, spores 14–17 × 4 μ) and the other species of *Exobasidium* described on Ternstroemiaceae appear to be confined to the leaves.

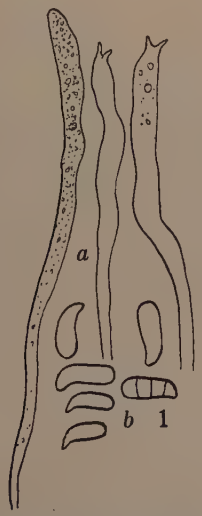


Fig. 1. *Exobasidium Camelliae* Shirai. *a*, basidia; *b*, spores (× 375).

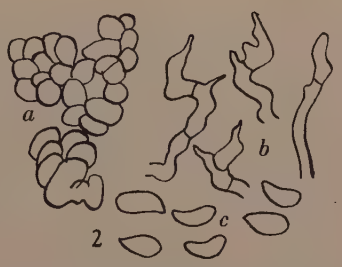


Fig. 2. *Herpobasidium filicinum* (Rostr.) Lind on *Dryopteris Filix-mas*. *a*, mycelium from three cells of host leaf; *b*, four basidia; *c*, six shed basidiospores (all × 300).

Herpobasidium filicinum (Rostrup) Lind in *Ark. Bot.* VII (8), 7 (1908).

Syn.: *Gloeosporium filicinum* Rostrup in *Thuem. Myc. Univ.* no. 2083 (1881).
Exobasidium Brevieri Boudier in *Bull. Soc. Mycol. Fr.* XVI, 15 (1900).

The fructifications of this fungus form white mould-like spots, mostly about 1 mm. across but sometimes confluent, on the lower surface of *Dryopteris* and *Polypodium* leaves. Corresponding small brownish angular spots appear on the upper surface of affected fronds. Infected host cells contain conspicuous coiled haustoria typical of the genus. The superficial mould consists of fine, hyaline, septate hyphae bearing terminal, two-celled basidia with stout sterigmata. The basidiospores are oval to oblong-apiculate, thin-walled, 18–24 × 6–9 μ, sometimes germinating *in situ* to produce secondary spores. These spore dimensions are a little greater

than those cited by Lind for his collections on *Dryopteris Filix-mas*, viz. 10–18 × 5–8 μ, basidia 40–50 × 9 μ, sterigmata up to 17 μ long.

British collections of this species are:

On *Dryopteris Filix-mas*, Breidden Hill, Montgomeryshire, 9 July 1942, N. Y. Sandwith; Harpenden, Hertfordshire, June 1931, A. Smith.

On *Polypodium Phegopteris*, Scotland, 25 June 1932, K. W. Braid.

It has been reported elsewhere on *Polypodium Dryopteris* and *Cystopteris montana*. According to Lind (op. cit.) Boudier's citation of *Athyrium Filix-femina* as a host of this fungus was an error. The cytology of this species has been fully investigated by Jackson (1935).

Puccinia commutata Sydow, *Monographia Uredinearum*, 1, 201 (1904).

P. Valerianae auctt. non Carest.

Aecidia developed on the lower surface of leaves, on petioles and on stems; on the leaves forming rounded or irregular groups; on stems arranged in more or less elongated series, seated on pale spots. Aecidiospores polygonal, finely warted, orange, 14–19 μ in diameter. Teleutosori hypophyllous, or on petioles or stems; on the leaves scattered or crowded, sometimes confluent, minute; on the stems generally densely crowded and confluent, forming more or less elongated pustules, pulverulent, dark brown. Teleutospores irregular, oblong, subfusoid or subclavate, moderately thickened at the apex, rounded or somewhat conical, attenuated with little or no constriction at the septum, narrowed at the base, rarely rounded, smooth, chestnut brown, 40–60 × 20–35 μ: pedicel rather long, hyaline, deciduous.

Fig. 3. *Puccinia commutata* Syd., teleutospores, apices swollen with potassium hydroxide (× 375).

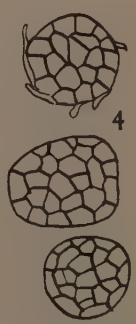


Fig. 4. *Tolyposporium Montiae* Rostr. from *Montia fontana*. Sporeballs (× c. 250).

On leaves and petioles of *Valeriana officinalis*, Loch Scridain, Mull, Argyllshire. Coll. T. A. Russell, 14 August 1928.

The aecidia and teleutosori occur intermixed, and on the petiole cause a certain amount of swelling and distortion. The species is widespread in Europe, but has frequently been referred to *Puccinia Valerianae* Carest., which is confined to *Valeriana celtica* and differs in the characters of the teleutospore.

Tolyposporium Montiae Rostrup (*Vejledn. i den danske Flora*, II, 31, 1904).
Sorosporium Montiae Rostrup in *Bot. Tidskr.* XX, 129 (1896).

Sori black; spore-balls subglobose or irregular, black, opaque, 45–60 μ in diameter, consisting of numerous spores. Spores irregular, polygonal, smooth, fuscous, not easily separating, 5–8 μ in diameter or 10 × 5 μ.

On leaves and stems of *Montia fontana*, West Kilbride, Ayrshire; D. A. Boyd, 3 June 1927.

The spore measurements given above are taken from the specimen in hand. In the original description the spore-balls are said to be somewhat larger, namely 60–80 μ. They are scattered throughout the lamina of the lower leaves, making them appear black to the naked eye and black-punctate under a lens. In the material to hand they are concentrated towards the leaf tips and scattered more sparsely towards their bases. Sori have not been seen on the stems.

Ustilago echinata Schroeter, *Abh. d. Schles. Ges. naturw. Abt.*, 1869, 4 (1871).

The species is omitted from the list of British smuts though possibly represented by Massee's reference to *U. longissima* (Schlecht.) Meyer on *Phalaris arundinacea* (Sampson, 1940). A leaf smut of *Digraphis arundinacea* presumably referable to *Ustilago echinata*, with spores 10–13 × 7–11 μ and very variable in shape, was collected in July and August 1943 at Bridge of Dun, Angus, and Castle Douglas, Kirkcudbrightshire. As this species seems to be doubtfully distinct from *U. striiformis* (Westend.) Niessl it will not be described more fully here. Fischer & Hirschhorn (1945), however, believe *U. echinata* can be separated from *U. striiformis* by its somewhat larger, rougher spores. At both localities other plants of this host in the same reed beds bore the head smut *Tilletia Menieri* Har. & Pat., already known from Ireland but not hitherto from Great Britain.

Lilliputia insigne (Winter) n.comb.

On 29 March 1938 specimens of a fungus on mushroom compost from Uxbridge, Middlesex, were received at Kew from Mr W. Buddin. The fructifications were spherical, glabrous, whitish to cream coloured, approximately 500 μ in diameter and scattered singly over the surface of the compost. Microtome sections showed them to have a stout wall, some 80 to 90 μ thick, composed of ten to twelve layers of cells, differentiated into an outer belt, three to four cells wide, of empty cells, and an inner zone, seven to eight cells wide, densely packed with oil globules. The centre of the ascocarp consisted of thin-walled tissue in which were embedded numerous asci, each with eight spherical yellow ascospores. The latter measured 11–13 μ in diameter with a large central oil globule and a wall about 1 μ thick, densely studded with short blunt warts. These measurements were made on spores within the asci.

Except for spore size this agrees precisely with the diagnosis and figures of *L. Gaillardii* described from humus in a glasshouse at Angers, France, April 1900. According to Boudier and Patouillard (1900) this fungus had spores 22–24 μ in diameter. Fortunately, type material of the species was

preserved by Patouillard and is now in the Farlow Herbarium. Dr Linder has kindly re-examined this and reports as follows: 'I have studied the type of *Lilliputia Gaillardii* Boud. & Pat. and find that the spores measure 13·2–18·2 × 18·2–24·5 μ. These spores are for the most part still within the ascus and tend to be somewhat compressed and therefore angular. In the few cases where the spores had been freed from the ascus the spores are perfectly globose, as stated by Patouillard.' It appears, therefore, that the spores of this fungus vary greatly in size and that the Angers and Uxbridge collections represent the same species. It is also the same as the fungus distributed under the name *Gliocladium penicilloides* Corda as number 120 of Reliquiae Farlowianae. This had been collected on decaying seaweeds at Kittery Pt., Maine, U.S.A., on 3 July 1918 by Thaxter, who cited as synonyms *Penicillium insigne* Bainier, *Licipenicillium insigne* Brefeld and *Lilliputia Gaillardii* Boud. & Pat. (Thaxter, 1922). Thom (1930), however,

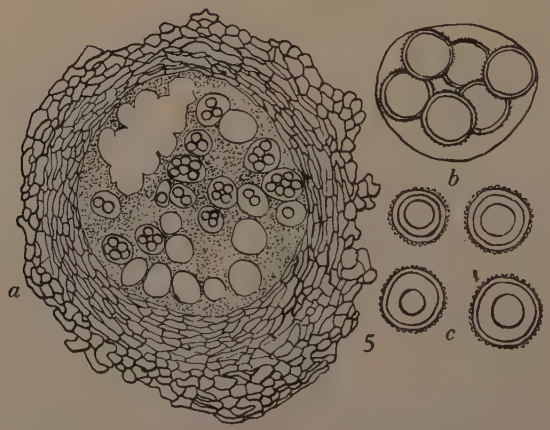


Fig. 5. *Lilliputia insigne* (Wint.) n.comb. *a*, microtome section of ascocarp from Middlesex (× 100); *b*, above: single ascus; below: two ascospores in optical section, from Middlesex material (× 750); *c*, ascospores from Rabenhorst, Fungi Europaei, no. 1732 (× 750).

regarded *Penicillium insigne* as probably distinct from *Gliocladium penicilloides* and the applicability of the latter name is also dubious as will be shown below.

Previously Winter (1873) had obtained an ascomycete on goose dung in Germany which he regarded as the perfect state of *G. penicilloides*. No description was published but material with adequate figures of asci and ascospores was distributed by Rabenhorst as Fungi Europaei, no. 1732, under the name *Eurotium insigne* G. Winter n.sp. (an n.g.?). Examination of Winter's material at Kew shows it to correspond closely with the present fungus except that the ascocarps are now a deep yellow-brown and the outermost cells appear deep yellow in section. The ascospores measure 13–15 μ across. Massee and Salmon (1901), who obtained *E. insigne* on dung of kangaroo, *Ovis burrhel*, fowl, and horse, at Kew described it as having the perithecia clear white when young, then pallid, becoming yellowish and finally rusty brown, with ascospores globose, 17–20 μ across.

The colour difference mentioned above is thus clearly not significant and depends probably on the mode of preservation. Winter's collection has been dried for over 70 years, the Uxbridge material has been kept in formalin.

These and other records of *Eurotium insigne* have been discussed by Petch (1939) who concluded that the imperfect fungus associated with it is not *Gliocladium penicilloides*, which he ascribes to *Hypomyces aureo-nitens* Tul., but *Gliocladium macropodinum* Marshal. The name adopted by Thaxter is thus inappropriate both on the ground of misidentification and of applicability only to an imperfect state. Though association with a *Gliocladium* was not noted in the Uxbridge material nor mentioned by Boudier and Patouillard, it appears at present highly probable that all the collections cited above are the same species. Owing to the very characteristic and distinctive ascospores it seems inadvisable to refer this fungus to *Eurotium*; it would appear preferable to retain the genus *Lilliputia*, with the new combination *L. insigne*. Boudier and Patouillard ascribed *Lilliputia* to the Tuberaceae, which is clearly inadmissible; Clements and Shear (1931) transferred it to Gymnoascaceae, in which it is equally out of place. Pending detailed studies of the developing ascocarp it seems to fall most naturally in Eurotiaceae, near *Eurotium*, from which it is distinguished by the spherical, warted ascospores.

Sphaerulina myrtillina Sacc. & Fautr. in *Bull. Soc. Mycol. Fr.* XVI, 21 (1900).

Spots amphigenous, reddish purple, rounded, 1–1·5 mm. in diameter, often confluent, eventually paler in the centre. Perithecia formed in the paler dead tissue, few, scattered, black, erumpent, 100–120 μ in diameter. Asci large, oblong or somewhat saccate, sessile obtuse, about 200 × 50–60 μ, eight-spored, paraphyses lacking. Spores more or less distichous, oblong, obtuse at both ends, finally tri-septate, slightly constricted at the median septum, hyaline, 45–50 × 15–18 μ.

On living stems of *Vaccinium Myrtillus*, Ben Ledi, Perthshire; W. B. Turrill, 1 September 1928.

The species is remarkable for the very large asci and spores. It has been removed by von Hoehnel to his genus *Pseudosphaeria*. As, however, doubt has been cast on the validity of the whole conception of the group Pseudosphaeriaceae, it seems at present best to retain the original name.

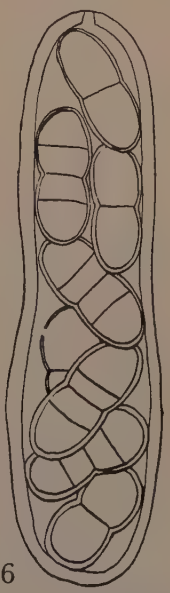


Fig. 6. *Sphaerulina myrtillina* Sacc. & Fautr. Ascus (× 375).

Sordaria caudata (Curr.) Sacc., *Syll.* I, 236.
Sphaeria caudata Currey in *Trans. Linn. Soc.* XXII, 320 (1859).

A small gathering of this apparently rare species was obtained from very soft, rotten wood in Rothiemurchus Forest, during the Aviemore Foray, 1927. Comparison with Currey's figure and with his specimen in the Kew Herbarium leaves no doubt as to its identity.

The perithecia are conical, very thin-walled and frequently collapsed, with the base sunken in the rotten wood. The spores, at first hyaline and cylindrical, at length develop a dark brown head, 17–20 × 9–10 μ, and a long, pale or hyaline tail, 30–32 × 4–5 μ. There is in addition a small, hyaline, pointed apical appendage, which is not shown in Currey's figure, but is present in his specimen and is also depicted by Berkeley and Broome (1871, tab. 21, fig. 25).

Leptospora caudata Fckl., *Symb. Mycol.* p. 144 (1869).

Perithecia superficial, gregarious, rather large, ovate, obtuse, rugulose, black, covered with short, multiseptate, dark, branched hairs; ostioles obtuse, perforate, subglabrous; asci long-stipitate, elongated, eight-spored, spore-bearing portion 128 μ long, 12 μ in diameter; spores oblong, obtuse at the apex, base oblique, acuminate-caudate, unseptate, multiguttulate, hyaline, 32 × 6 μ.

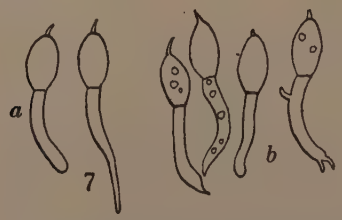


Fig. 7. *Sordaria caudata* Currey. *a*, two ascospores from type specimen; *b*, four ascospores from Aviemore specimen (× 375).

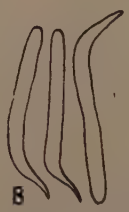


Fig. 8. *Leptospora caudata* Fckl., from Tintern. Ascospores (× 600).

The above is a translation of Fuckel's description. At the Tintern Spring Foray there was collected a Pyrenomycete which apparently belongs to the above species. Unfortunately, the material is so scanty that it is impossible to say whether it agrees with the description in all details. The perithecia in general appearance suggest *L. spermoides*, but are more rough. A covering of branched hairs is not now present, but as many of the perithecia are empty it is obvious that the material is old, and such a character may well be evanescent. The characteristic feature lies in the peculiar spores. In the Tintern specimen they are exactly as described by Fuckel, hyaline, unseptate, with a knee-like bend at the base terminating in a slender 'tail'. They measure 35–40 × 4 μ. Asci not seen.

On dead larch twig, Tintern, May 1925.

Under the manuscript name of '*Sphaeria falcispora* Broome' there is in the Kew Herbarium a specimen with similarly shaped spores. The perithecia in this material are at first distinctly hairy, becoming smooth with age, and in this respect the fungus would agree with Fuckel's description. The spores, however, are longer even than those of the Tintern specimen, being 67–70 × 5 μ.

Nectriella Lophocoleae Massal. in *Mem. dell' Acc. delle Sci. Med. e Nat. di Ferrara*, 5 Magg. 1895.

This interesting fungus was described from living leaves of the liverwort

Lophocolea cuspidata near Massa Conora, Italy. The diagnosis runs as follows: Perithecia superficial, solitary to subaggregated, pale orange, ovoid, seated on a light coloured filamentous subiculum, 120–150 μ in diameter, covered with bristly setae, setae continuous, thick-walled, simple, 8–10 μ thick, about half the length of the perithecium, ostiole apical, indistinct, wall membranaceous; asci cylindrical clavate, rather long pedicillate-stipitate, fertile portion 70–110 × 16–22 μ. Spores eight, ellipsoidal, 22–30 × 10–14 μ, hyaline, rounded at the ends, guttulate; paraphyses numerous, filiform, 1–1·5 μ thick, as long as the asci, sometimes branched.

Fig. 9. *Sphaeria falcispora* Broome. Two ascospores, from specimen in Herb. Kew. (× 600).

A few specimens of this species were collected by Miss M. Buchanan on *L. bidentata* at Dargle near Dublin, Éire, in March 1929, and sent to Kew by Miss M. C. Knowles. The perithecia were reddish brown, covered with subhyaline blunt setae, up to

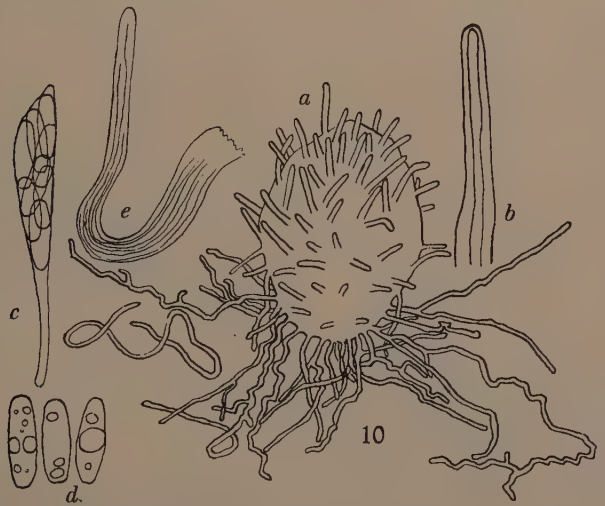


Fig. 10. *Nectriella Lophocoleae* Massal. *a*, perithecium (× 60); *b*, seta from same perithecium (× 300); *c*, undischarged ascus; *d*, three discharged ascospores (× 300); *e*, empty ascus (× 300).

c. 140 μ long and 8–9 μ thick. Asci and paraphyses were freely liberated from the apex of the perithecium by slight pressure on the cover slip and the spores appeared to emerge from the asci in groups of eight, as though still enclosed in a fine membrane. They measured 34–40 × 12–13 μ. In Petch's (1938) classification this species appears to be a *Pseudonectria*.

Rhytisma symmetricum J. Müller in *Pringsh. Jahrb. f. wiss. Bot.* xxv, 622 (1893).

Spots numerous on each leaf, black, irregular in shape, smaller than in *R. salicinum*. Stromata extending right through the leaf and equally prominent on either side, 1–3 mm. in diameter. Apothecia amphigenous. Asci 135–162 × 12–19 μ; spores filiform, 30–108 μ long. Paraphyses short, slightly enlarged above.

The measurements of asci and spores given are from the original description. The material sent was immature, but the species is easily distinguished from *R. salicinum* by the numerous small spots on the leaf, and especially by the symmetrical development of the stromata on both sides of the leaf.

On leaves of *Salix*, Great Yarmouth. Received from Dr G. H. Pethybridge, October 1926.

A new *Sclerotinia* on *Gladiolus*

In the autumn of 1938 Mr W. Buddin was working on the diseases of *Gladiolus* corms, and isolated what appeared to be two different species of *Botrytis*. One, the more common, was of the *B. cinerea* type, with sclerotia, but these not very abundant. The other, with more narrowly elliptical conidia, produced abundant sclerotia aggregated in large masses, which in appearance suggested those of *B. convoluta* Drayton, described from *Iris*. They were, however, not truly convolute. Using the technique described by Drayton (1934) for *Sclerotinia Gladioli*, Mr Buddin was successful in producing by spermatization the apothecia of this form, and brought material to Kew in the early part of 1939. As might have been expected, the apothecia and spores do not differ greatly from those of other species included by Whetzel in his genus *Botryotinia* (i.e. species of *Sclerotinia* having a conidial stage of the *Botrytis cinerea* type), but the distinctive characters of the conidia and sclerotia distinguish this fungus from any other so far known on *Gladiolus*, and appear to warrant the establishment of a new species. Circumstances have prevented the writing up of the whole of the work, and it seems advisable now to publish the diagnosis of the new species, so that it may be available for other workers in the same field. The authors have given the specific epithet in honour of Dr F. L. Drayton of Ottawa, whose unfailing interest and generosity in connexion with the work has been of much encouragement.

Sclerotinia Draytoni Buddin & Wakef., sp.nov.

Sclerotia atra, laevia, applanata demum convexa (lenticularia), circa 8–12 × 3–7 mm., in massis irregularibus usque ad 2·5 cm. longis agglutinata, intus albida, ex hyphis hyalinis crasse tunicatis arcte intertextis composita. Micro- et macroconidia adsunt. Microconidia globosa, 2–2·5 μ diam. Macroconidiophora e sclerotiis oriunda, erecta, deorsum fusca, 12–15 μ diam., sursum hyalina, ramosa. Rami 2–3, obtusi, interdum furcati, plerumque sursum capitato-incrassati, sterigmatibus minutis deciduis instructi. Conidia hyalina vel pallide fusca, cylindrico-ellipsoidea vel anguste ovata, 8–16 × 5–7·5 μ. Apothecia longe stipitata, singulatim vel gregatim e sclerotiis enascentia, primo infundibuliformia margine incurvata, dein umbilicato-discoidea, denique leviter convexa, margine reflexa. Hymenium 2·5–5 mm. diametro, ex argillaceo fulvo-olivaceum margine primo brunnea, demum pallidiore, demum concolor. Stipes tenuis, elongatus, 10–13 mm. longus, flexuosus, sursum incrassatus, hymenio concolor, basim versus fuscescens, plerumque glaber sed aliquando ob conditiones humidos pilosus. Asci cylindrici, octospori, 140–190 μ longi, 7·5–9 (–10) μ crassi,

pars sporifera circa 80 μ longa. Ascosporae monostichae, anguste ellipsoideae vel subfusiformes, hyalinae, saepe biguttulatae, 12–17 × 6–8 μ (medio 15 × 6 μ). Paraphyses filiformes, apice clavato incrassatae.

Hab. In caulibus Gladioli sp. cult., parasitica.

Sclerotinia hirtella Boudier, *Icon. Mycol.* III, tab. 471 (1907).

Two specimens which appear referable to this species were found on 29 May 1945 arising from sclerotia attached to fallen bud scales of some deciduous tree lying in a damp situation under a clump of rushes on Leith

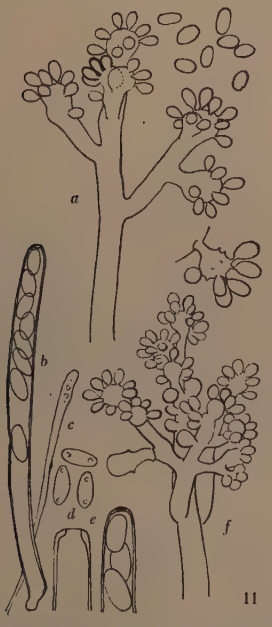


Fig. 11. *Sclerotinia Draytoni* Buddin & Wakef. *a*, conidiophore (× 400) and conidia (× 250); *b*, ascus; *c*, paraphysis; *d*, three spores (× *c.* 325); *e*, apices of two asci (× 500); *f*, conidiophore (× 250).

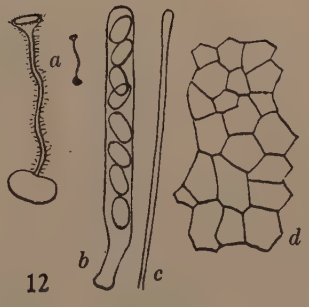


Fig. 12. *Sclerotinia hirtella* Boud. *a*, sclerotium and ascocarp, nat. size and × 3·75; *b*, ascus; *c*, paraphysis; *d*, superficial cells of sclerotium (× 450).

Hill, Surrey. Boudier's collection was on rotting sweet chestnut twigs but it is impossible to say whether this holds for the Surrey material or not, though sweet chestnut is plentiful in the vicinity. Boudier's diagnosis is as follows: Apothecia minute, slender, stipitate, 5–10 mm. high, entirely pale ochraceous-cinereous, hairy, arising from a small, black oblong sclerotium. Receptacle at first cup-shaped, ultimately flattened, externally covered, like the stipe, with long, flexuous septate, pointed, colourless hairs, 90–110 μ long × 4 μ thick, and simple or bifid. Hymenium smooth and concolorous, paraphyses linear, colourless, septate, slightly thickened to about 3 μ at the tip; asci cylindrical, very slightly attenuated at the base, eight-spored, inoperculate, 90–115 μ long by 7–8 μ wide; spores oval-oblong, colourless, non-guttulate, 7–9 × 4–5 μ.

Ascotremella faginea (Peck) Seaver in *Mycologia*, XXII, 53 (1930).
Haematomyces fagineus Peck in *Ann. Rep. N.Y. State Mus.* XLIII, 33, 1890.

In August 1944 Mr A. A. Pearson sent to Kew a fungus which proved to be of considerable interest. At first sight it suggested the conidial stage of *Coryne sarcoides*, but was more cerebriform and of a rather duller colour. On microscopic examination it was found to be an Ascomycete (Discomycete) but without distinct cup-like apothecia, and the spores were quite different from those of *Coryne*. The only species found in literature to which it can be referred is *Ascotremella faginea* (Peck) Seaver. In the article referred to in *Mycologia*, Seaver has shown that the generic name *Haematomyces* cannot be used for this fungus. The original species on which the genus *Haematomyces* was founded has been shown to be a resinous exudation, not a fungus at all. Later authors have included in the genus, Ascomycetes which are not congeneric, and it would therefore be best to abandon the name *Haematomyces* altogether. For these plants which are tremelloid in habit but have asci with hyaline unseptate spores, Seaver has proposed the new name *Ascotremella* designating *A. faginea* as the type species.

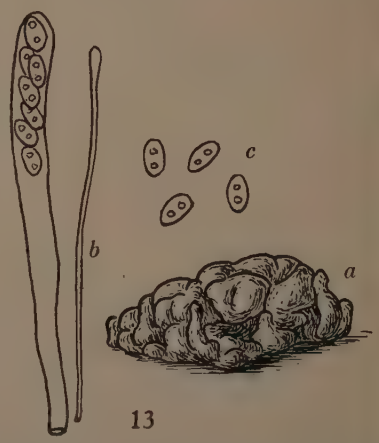


Fig. 13. *Ascotremella faginea* (Peck) Seaver. *a*, Ascoma (nat. size); *b*, ascus and paraphysis (×600); *c*, spores (×600).

Mr Pearson's plant agrees on the whole very well with the description given by Seaver, which was apparently compiled from dried material and photographs. The colour, however, of the British plant is rather more purplish than is suggested by the description 'raisin-coloured', the asci are longer and the spores tend to be subdistichous rather than clearly monostichous (the description says 'usually one-seriate'). The following description was drawn up from the fresh plant:

Ascoma tremelloid, cerebriform, gelatinous, smooth, dull reddish purple in colour when fresh and about 4 cm. in diameter, but shrinking when dry to a hard, horny mass less than half that size. Asci covering the whole lobed outer surface, cylindrical, up to 95 μ long, 7 μ broad above, inoperculate, spores occupying less than half the total length, subdistichous, eight in number. Spores elliptical, hyaline, with two oil-drops, thin-walled, (6–)8–10 × 4 μ. Paraphyses slender, hyaline, slightly thickened towards the apex. Hyphae of hypothecium 2–6 μ, embedded in a gelatinous matrix.

On dead wood, West Dean Wood, near Goodwood, Sussex. Coll. A. A. Pearson, August 1944.

Protomyces inundatus Dangeard in *Le Botaniste*, IX, 274 (1906).

After pointing out that on most hosts the sporangia of *Protomyces macrosporus* Ung. germinate by rupture of the epispore, followed by emergence of the contents still surrounded by endospore and mesospore before sporu-

lation, Dangeard remarked that in the race on *Helosciadium nodiflorum* the spores form in the interior of the unbroken sporangium. He continued: 'Must we found a new species, characterized by this mode of germination? We think not, and it is simpler, we believe, to attribute this difference in mode of germination to the difference in habitat. When the parasite lives on a terrestrial umbelliferous host the chlamydospores extrude their sporangia to the exterior, when, as here, it is on an aquatic host, germination occurs under different conditions in that the host is totally or partly immersed. If, however, one wishes to establish a new species we propose the name *Protomyces inundatus*.' von Büren (1922) showed experimentally that *P. inundatus* on *Helosciadium nodiflorum* could not be transferred experimentally to *Aegopodium Podagraria*, *Sium erectum*, *Apium graveolens* or *Helosciadium inundatum*. In view of this close biological specialization he accepts it as a species distinct from *Protomyces macrosporus*. He retains it, however, in *Protomyces*, in contrast to his earlier view (v. Büren, 1915) that the fungus on *Helosciadium nodiflorum* was a *Taphridium*. The name *Protomyces inundatus* Dangeard was accordingly compiled in Saccardo, vol. xxvi, but no formal diagnosis of the species has been traced.

P. inundatus is not uncommon on *Helosciadium nodiflorum* in Britain. Collections exist at Kew from Reading, 6 July 1943, L. Hawker; Farlington Marshes near Plymouth, 24 August 1945, J. Staley; Conway, 27 June 1927, W. B. Grove; Cefn, Beaumaris, Anglesey, 29 July 1927, P. G. M. Rhodes; Wirral, 22 December 1912, J. W. Ellis; Scarborough, 7 August 1911, T. B. Roe. A collection from Shrewsbury, July 1883, which appears to be the same fungus, is filed under the manuscript name *Protomyces Helosciadii* Phil.

Pseudoperonospora Erodii (Fuckel) G. W. Wilson in *Mycologia*, vi, 194 (1914).

Syn. *Peronospora Erodii* Fuckel, Fungi Rhenani, no. 2102 (1868).
Plasmopara Erodii (Fuckel) D. Sacc. *Mycoth. Ital.* no. 890.

This rather inconspicuous downy mildew was found associated with small brown lesions up to 5 mm. long on living leaves of *Erodium cicutarium*, near Dorking, Surrey, 3 June 1945. The conidiophores form dense, greyish violet clusters on the lower surface of the leaf, about 100–300 μ long, 4–6 times dichotomously branched, with the main stem occupying from one-half to two-thirds of the total length, 8–10 μ thick, swelling to 13 μ at the base, and terminal branches 5–10 μ long, slightly curved. Conidia are yellow-brown and vary from almost globose bodies 16–23 μ across to ovoid ones 23–35 × 18–24 μ.

Peronospora Gei Sydow in Gaumann, *Beitr. Krypt. Flora der Schweiz*, v, Heft 4, 291 (1923).

On 13 June 1945 Mr H. K. Airy Shaw collected leaves of cultivated *Geum*, varieties Mrs Bradshaw and Lady Stratheden, in the Rectory garden at Daglingworth, Glos., heavily infected by *P. Gei*. Many plants showed the lower surface of the larger leaves almost completely covered with a greyish coating of conidiophores and conidia; in others sections of the

lamina delimited by the main lateral veins were similarly affected. Corresponding chlorotic patches on the upper surface of the leaves rendered the diseased plants extremely conspicuous. It is the more remarkable that this destructive disease seems to have hitherto remained unrecorded in Britain. The diagnosis of *P. Gei* follows: Conidiophores densely crowded, in a greyish white felt, covering parts of the lower surface of the leaf;

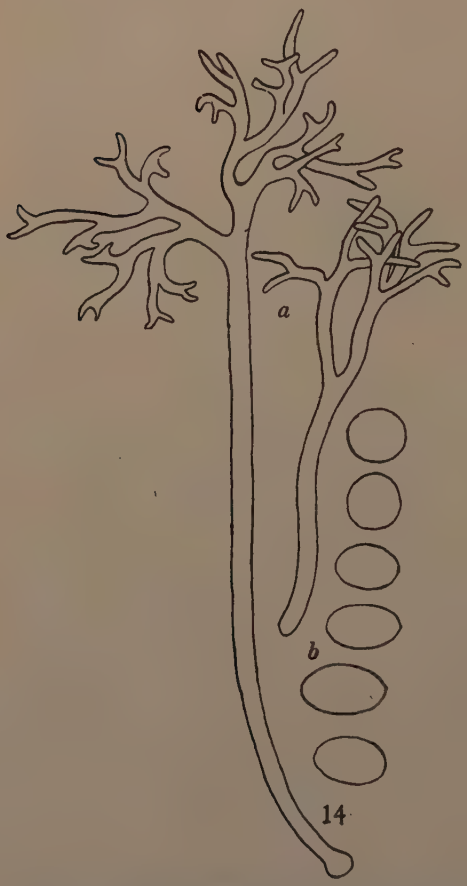


Fig. 14. *Pseudoperonospora Erodii* (Fckl.) G. W. Wilson. *a*, two sporophores; *b*, six conidia (×300).

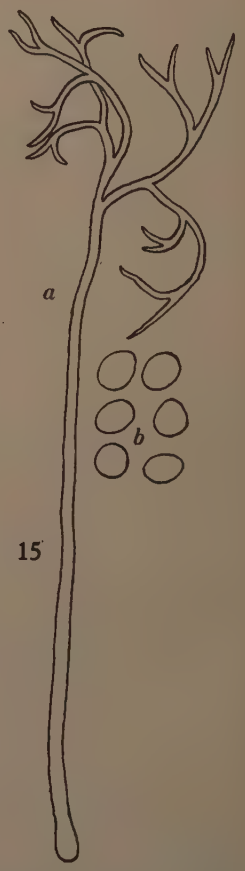


Fig. 15. *Peronospora Gei* Syd. on cultivated *Geum*. *a*, sporophore; *b*, six conidia (×300).

commonly extruded in groups from the stomata, 200–500 μ long, the main axis forming one-half to two-thirds of the total length, 4–8 μ thick, base slightly swollen, four to six times dichotomously branched, branches spreading, undulating; terminal branches 10–30 μ long, much curved. Conidia light brown, broadly ellipsoidal, 8–24, mostly 15–19 μ long, 8–21, mostly 13–18 μ broad; mean size 17·09 × 15·26 μ. Oospores unknown. The type collection was apparently on *Geum album* from North America,

but Gaumann regarded forms on *G. rivale* and *G. urbanum* as belonging to the same species, probably also those on *G. coccineum* and *G. macrophyllum*. *G. urbanum* in the vicinity of the diseased garden plants at Daglingworth remained healthy.

Two fungi of apple leaf spots

In September 1944 we received from Dr E. Gray of the north of Scotland Agricultural College, Aberdeen, three apple leaves from Achnashellach, Ross-shire, marked by more or less circular, whitish spots of dead tissue, between 2 and 6 mm. in diameter. Each spot was sharply defined by a narrow dark brown line and bore on its upper surface numerous (up to 90) minute black pycnidia, just visible to the unaided eye. Examination showed that though the pycnidia appeared uniform externally, even from a single spot some yielded hyaline, non-septate, somewhat fusiform and usually biguttulate spores measuring $2{\cdot}5$–6×2–$4\,\mu$, mostly about $5 \times 3\,\mu$, while others contained very pale brown, elliptical, non-guttulate spores measuring 4–5×2–$3\,\mu$. The former was tentatively identified with *Phyllosticta Mali* Prill. & Del., the latter with *Coniothyrium tirolense* Bub. not hitherto reported from Britain. Cultures from spores of the *Coniothyrium* type yielded a thin brown mycelial mat with scattered clusters of black pycnidia which contained typical *Coniothyrium* spores 3–5×2–$2{\cdot}5\,\mu$ with a mean size of $4{\cdot}3 \times 2{\cdot}8\,\mu$. No lesions were induced by this fungus on Doon Star potato tubers, but on apple fruit it caused small, round, superficial, blackish lesions and on tomato fruit it gave rise to a rather slow rot which attained a diameter of 14 mm. in 8 days at room temperature. All inoculations were made by inserting a fragment of malt agar culture into a minute slit in the surface of fruit or tuber concerned.

According to Wollenweber and Hochapfel (1937) *C. tirolense* has persistently light brown pycnospores of mean size $5{\cdot}6 \times 3\,\mu$, causes hard, leathery, black spots on apple fruit, and is able to rot tomatoes and pears. The identification of the Scottish fungus with that species was therefore upheld by the inoculation experiments described above. There is no material of *C. tirolense* under that name at Kew but a collection of *C. piricola* Potebnia, distributed by Savulescu as Herb. Mycolog. Romanicum, no. 1171 and preserved in the herbarium of the Imperial Mycological Institute, yielded similar spores 3–$7{\cdot}5 \times 2$–$3{\cdot}5\,\mu$, mean size $5{\cdot}7 \times 2{\cdot}9\,\mu$. It thus agreed with Wollenweber and Hochapfel's conception of *C. tirolense*, of which they regard *C. piricola* as a synonym. A further specimen in the same herbarium, collected on 'crab' apple at Indian Head, Saskatchewan, which had spores 4–$6 \times 2{\cdot}5$–$3{\cdot}5\,\mu$, mean size $5{\cdot}1 \times 3{\cdot}0$, had been determined by Dr G. R. Bisby as *C. pirinum* (Sacc.) Sheldon. The spots appeared identical on the leaves of all these collections and though the spores of the Scottish material are a shade smaller than the others there seems no reason to doubt that all three represent the same species.

Owing to its presence on the same small spots it was natural to suppose that *Phyllosticta Mali* might be an early stage in the development of this fungus, but, owing to the difference in shape as well as colour of the spores, it seemed desirable to check this attribution by artificial cultures. Single

pycnidia of the *Phyllosticta* and of the *Coniothyrium* were therefore cut out of the spots and crushed in sterile water and cultures were started from the resultant spore suspensions on maize meal agar on 14 April 1945. The spores of both fungi were still freely viable although the leaves had been stored dry in a herbarium capsule for seven months. Eleven isolations from the same pycnidium of *Phyllosticta Mali* yielded apparently identical cultures of a rather quick-growing fungus which produced numerous, scattered, yellow-brown pycnidia within four days at room temperature. After a fortnight's growth there were still no aerial hyphae and no dark colour in the submerged mycelium so that the pycnidia formed almost the only features visible when the plate was held up to the light. On malt agar slants, there was a profuse development of short, dense, whitish to pinkish brown aerial mycelium passing into a greenish brown zone near the top of the slant. Eight isolations from a single pycnidium of the *Coniothyrium* yielded a rather slower growing fungus which produced a dense clump of aerial mycelium round the points of inoculation. This mat, at first white, rapidly turned grey and after a fortnight's growth each isolate consisted of a central dome of grey aerial hyphae surrounded by a more or less distinct, short, grey-brown mat, succeeded by a broad marginal belt free from aerial mycelium. Black pycnidia were scattered sparsely, singly and in small groups, over the central portion of each mat. Mycelial growth on malt agar slants was much more copious and scarcely distinguishable from that of the *Phyllosticta*, the aerial mat was, however, darker brown than in the latter fungus and pycnidia were far less abundant.

The *Phyllosticta* pycnidia produced in culture were approximately 200–250 μ in diameter, with one or often two well-defined ostioles, and bore a pink spore exudate consisting of hyaline spores, similar in shape to those produced on the host but less distinctly guttulate, and measuring 4–6 × 2·5–3 μ, mean size 4·5 × 2·7 μ. The *Coniothyrium* pycnidia were similar in size but from the first yielded yellow-brown spores 3–4 × 2·5–3 μ, mean size 3·2 × 2·7 μ. The two fungi were therefore clearly distinct and did not belong to the same life cycle.

Reference to the literature shows that similar observations were made on apple leaf-spot fungi by Crabill (1912) in Virginia in 1911. The *Phyllosticta* described above agrees fairly well with his strain 2, identified as *P. pirina* Sacc. while the *Coniothyrium* seems to be his conception of *C. pirinum* Sheldon. The question therefore arises, what are the correct names of these two fungi?

The name *Phyllosticta pirina* was applied by Saccardo (1878) to a pycnidial fungus of pear and apple leaves described as having ovoid to ellipsoid hyaline spores 4–5 × 2–2·5 μ. The type is apparently preserved in Saccardo's herbarium at Padua (Gola, 1930) and without reference to it, it is scarcely possible to be certain what his fungus was, but the absence of any reference to guttules and the description of spore shape suggests an early state of the *Coniothyrium* rather than the *Phyllosticta* described above. Sheldon (1907), in West Virginia, collected a *Coniothyrium* on apple and quince leaves which he identified with Saccardo's fungus, and accordingly renamed the latter *C. pirina* (Sacc.) Sheldon. 'Specimens of the fungus on

apple and quince leaves were submitted', to Saccardo, 'for determination and comparison with type specimens of *Phyllosticta pirina* Sacc.' Presumably Saccardo accepted the identification though Sheldon does not precisely say so. Meanwhile Bubák (in Bubák and Kabát, 1904) had described *Coniothyrium tirolense* as a parasite of living pear leaves in Tyrol, in association with *Phyllosticta tirolensis* Bub. Three years later Potebnia (1907) published the name *Coniothyrium piricola* for a similar fungus on apple leaves in Russia, associated with a *Phyllosticta* identified as *P. Briardi* Sacc. Wollenweber and Hochapfel (1937) reduced *Coniothyrium piricola* to a synonym of *C. tirolense* and, assuming Sheldon's identification to be correct, there seems every reason to regard both names as synonyms of *C. pirina.*

When Crabill demonstrated the occurrence of a *Phyllosticta* distinct from *Coniothyrium pirinum* on apple leaf spots in Virginia he adopted the names *Phyllosticta pirina* Sacc. and *Coniothyrium pirina* Sheldon but as Sheldon's name originated in a transfer of Saccardo's specific epithet from *Phyllosticta* to *Coniothyrium* and the same type collection can hardly be held to be the type of two different species these names would seem to be inadmissible. Meanwhile, Prillieux and Delacroix (1890) had described a *Phyllosticta Mali* on apple leaves at Lorient, France, which seems clearly to be the *Phyllosticta* both of Crabill and of the Scottish material. Though their meagre diagnosis merely refers to 'sporulis ovoideis 6·5–8·5 × 4–4·5 μ' their figure distinctly shows rather fusiform biguttulate spores and theirs would seem to be the earliest name that can with confidence be applied to the species in question. *P. Briardi* Sacc. published in June 1892 may be the same fungus but is described as having smaller spores and as being associated with a different type of leaf spot. Salmon (1907) reported *P. Mali* on Peasgood Nonsuch, Cox's Orange Pippin and Ribston Pippin apple leaves in England. His figures show precisely the same type of spot as that dealt with here and also the typical, biguttulate, *Phyllosticta* spores. He made no mention of a *Coniothyrium.*

In the meantime the apple leaf-spot fungi may therefore be named:

(1) *Phyllosticta Mali* Prill. & Del. 1890.

Syn. *P. tirolensis* Bub. 1904 (probably).

(2) *Coniothyrium pirinum* (Sacc.) Sheldon, 1907.

Syn. *Phyllosticta pirina* Sacc. 1878.
Coniothyrium tirolense Bubák, 1904.
C. piricola Potebnia, 1907.

No great reliance can be placed on either name, however, as it seems most unlikely that either fungus is confined to leaves of apple and pear, or even to *Pyrus* leaves in general. Wollenweber and Hochapfel have already obtained *Coniothyrium tirolense* from dry twigs of *Juglans mandschurica* in the botanic garden, Berlin-Dahlem. It is quite likely that when adequate cultural studies of the Sphaeropsidales can be undertaken, each species will be found to have many other synonyms applied to fungi collected on other substrata, some of which may well antedate the names suggested above.

Crabill found both species to be non-parasitic to uninjured apple leaves; in his view they were saprophytes or facultative parasites on lesions initiated by *Sphaeropsis malorum*. It does not follow that the same condition obtains in Britain, as Crabill's fig. 18 of diseased apple leaves shows a 'frog-eye' type of spot with very broad brown border. This evidently represents a more serious disease than that represented by the Scottish material, on which there is no trace of a *Sphaeropsis*.

Thanks are due to the Director of the Imperial Mycological Institute for kindly providing the facilities which made continuation of this work possible after the junior writer's transference to Kew.

Strasseria carpophila Bres. & Sacc.

The occurrence of this fungus on black rotted apples in Edinburgh has already been reported elsewhere (Dennis, 1943).

Septocylindrium Aspidii Bresadola in *Hedwigia*, xxxv, 201 (1896).

Septocylindrium Aspidii forms a wide-spreading powdery white bloom on the upper surface of *Aspidium* fronds. The conidiophores are short, 6–8 μ long, 3 μ broad; the conidia cylindrical, straight or slightly curved, 15–24 $\times$ 3 μ, multiguttulate, with one- to four-septa. The above diagnosis is based on that given by Bresadola; spores from a British collection on *Aspidium* sp., woods between Ceres and Cupar, Fife, 5 July 1944, measured 14–33 $\times$ 2·5–3 μ but were not clearly septate. The disease was at that time widespread in East Fife and, as Bresadola observed, caused no well-defined spots but formed a conspicuous white bloom over extensive dead portions of the pinnae.

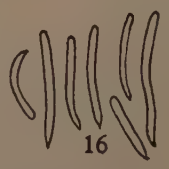


Fig. 16. *Septocylindrium Aspidii* Bres. Conidia (× *c.* 550).

Dactylium dendroides Fr. subsp. *leptosporum* Sacc. in *Michelia*, II, 576, 1882.

The following description is based on growth in a malt agar culture of a fungus isolated by Dr W. A. Millard from diseased bracken fronds on a moor in Yorkshire, in September 1945.

Submerged hyphae hyaline, about 2–6 μ in diameter, rather sparingly septate; aerial hyphae forming a rather loose, short, buff-coloured turf. Conidiophores not clearly differentiated from the mycelium. 300 μ or more long, about 5 μ thick at the base, tapering to about 3 μ at the apex, sparingly septate, bearing up to four whorls of phialides, 3–6 (usually 3–4) in a whorl, individual phialides occasionally growing out to form branches with secondary whorls. Conidia dry, hyaline, smooth, oblong-elliptical, equally rounded at each end or slightly narrowed to the base, marked by a small apiculus, three-septate at maturity, 18–24 $\times$ 5–6 μ.

These conidia appear to be somewhat narrower than those seen by Saccardo, whose diagnosis was: 'Conidiis paulo angustioribus, nempe 22 $\times$ 8 μ oblongis, 3-septatis, basi apiculatis, hyalinis. In corticibus emor-

tuis udis Newfield, N. J.' In view of this discrepancy and the brevity of Saccardo's description, the identification of this fungus must be regarded with some reserve.

Fig. 17. *Dactylium dendroides* subsp. *leptosporum* Sacc., from malt agar culture one month old. *a*, terminal portion of two conidiophores; *b*, four detached conidia (×400).

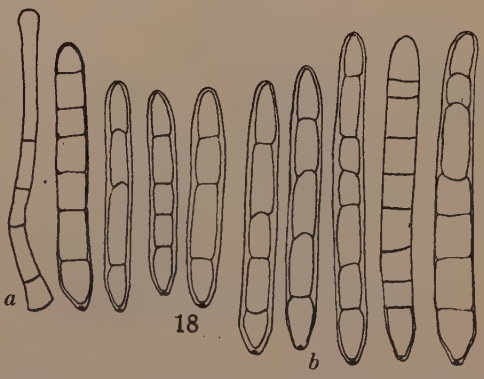


Fig. 18. *Helminthosporium tritici-repentis* Died. *a*, conidiophore; *b*, nine conidia from dead leaf of *Agropyron repens*, Kew (×300).

Helminthosporium tritici-repentis Diedicke ex Drechsler in *Journ. Agric. Res.* XXIV, 667 (1923).

The study of graminicolous species of *Helminthosporium* has been sadly neglected in Britain. There follow diagnoses of a number of species recorded, apparently for the first time in England, in the summer of 1945.

H. tritici-repentis has dark olivaceous conidiophores, 80–220 × 7–9 μ, three- to six-septate, with a swollen basal segment; conidia subhyaline, straight cylindrical, one to nine times septate, 45–175 × 12–21 μ (Kew collection 98–160 × 12–18 μ, two- to eight-septate). The basal segment of the spore tapers abruptly to the hilum, at which it becomes rounded off; the resulting profile recalls the head of a snake seen from above. The distal segment is usually rounded off in a hemispherical form but the two or three terminal segments may taper appreciably towards the distal end.

On dead basal leaves of *Agropyron repens*, without any distinctive lesions, Kew, 24 May 1945.

Helminthosporium dictyoides Drechsler in *Journ. Agric. Res.* XXIV, 679 (1923).

Conidiophores dark brown or olivaceous, 70–150 × 6–8 μ, three- to six-septate, geniculate; conidia subhyaline, later yellow, typically straight

with maximum diameter at the basal segment, 14–17 μ, tapering uniformly and very perceptibly to the apical segment, which in long spores often does not exceed 8–9 μ in diameter; less often approximately cylindrical or short ellipsoidal; length 23–115 μ, usually 50–70 μ, one- to seven-septate, typically three- to five-septate, not perceptibly constricted at the septa. Germination is typically by two germ tubes, one from each end segment, and produced usually at a right or oblique angle to the axis of the spore. Newly infected leaves show irregular brownish areas marked with dark longitudinal and transverse streaks, forming a delicate reticulate pattern.

The above diagnosis is condensed from that of Drechsler for a fungus on *Festuca elatior*. What appears to be the same species occurs at Kew on *Lolium* with conidia 60–150 μ, mainly 75–105 μ long, basal cell 13–18 μ wide, apical cell 10–14 μ wide at the septum. It seems to be distinguishable from *Helminthosporium siccans* on the same host by the tapering spores, characteristic net blotch lesions and mode of germination, but the relationships of these two fungi obviously call for investigation.

Helminthosporium dematioideum Bubák & Wróblewski in *Hedwigia*, LVII, 337 (1916).

The brief original diagnosis of this species ran as follows: fructifications minute, scattered, pulverulent, black, mycelium dematioid, blackish brown. Conidiophores cylindrical, 25–60 μ long, 5–6 μ broad, subtorulose, septate, brown. Conidia cylindric-oblong, 39–42 μ long by 9–13 μ broad, three-septate at maturity, rounded at each end, yellow-brown, thick-walled, smooth, on glumes and pales of *Anthoxanthum odoratum*. Drechsler (1923) identified with this a fungus encountered in America on fading leaves of the same plant, having characteristically slender conidiophores and two- to six-septate spores 18–48 μ × 8·5–14 μ. What is clearly the same species was found on dead basal leaves of *A. odoratum* on Leith Hill, Dorking, 3 June 1945, with conidiophores 50–90 × 6 μ and one- to four-septate spores 30–50 × 9–16 μ.

Helminthosporium triseptatum Drechsler in *Journ. Agric. Res.* XXIV, 686 (1923).

Fructifications scattered sparsely on withering leaves of *Holcus lanatus*, not associated with visible lesions. Sporophores arising singly or in pairs, 200–400 × 6–8 μ, usually six- to eleven-septate; proliferation of spores is associated with conspicuous local thickening of the peripheral wall, resembling a ring or band: these thickenings occurring in series give the upper portions of the sporophores a more or less moniliform contour. Spores dark olivaceous, ellipsoidal or short cylindrical with hemispherical ends, sometimes tapering somewhat towards basal end, regularly two- to three-septate, with an unusually thick peripheral wall, not contracted at the septa; 35–50 × 15–21 μ (Surrey collection 34–45 × 15–20 μ). Germination is by production of one or two germ tubes at positions adjacent to the hilum.

The type host is *Holcus lanatus* and the fungus occurred on dead basal leaves of that grass at Hackhurst Downs, Surrey, 17 June 1945. It was

associated with another species having cylindrical to fusoid, four- to eleven-septate conidia 56–135 (–230) × 10–16 μ, and bipolar germination. Of the graminicolous forms this seems nearest to *H. Leersii* but may well be an undescribed species. Drechsler (1935) ascribed a minor leaf spot of *Agrostis* spp. to *Helminthosporium triseptatum.*

Fig. 19. *Helminthosporium dictyoides* Drechsl. *a*, nine conidia, one germinated after 4½ hours in tap water (× 300); *b*, net-blotch lesion on lower surface of *Lolium* leaf, yellow area stippled, necrotic areas in black. (× *c.* 4).

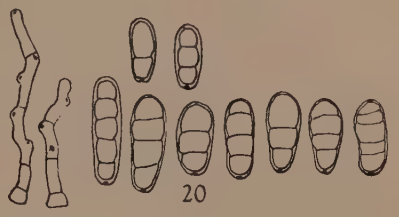


Fig. 20. *Helminthosporium dematioideum* Bub. & Wróbl. Conidiophores and conidia from faded leaf of *Anthoxanthum odoratum* (× 300).

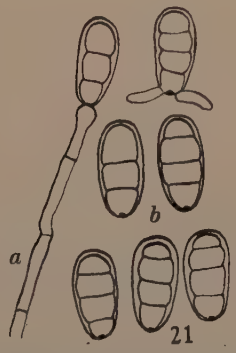


Fig. 21. *Helminthosporium triseptatum* Drechsl. *a*, conidiophore; *b*, six conidia, one germinated in tap water, from dead leaves of *Holcus lanatus* (× 300).

Helminthosporium stenacrum Drechsler in *Journ. Agric. Res.* XXIV, 683 (1923).

Sporophores solitary or in pairs, geniculate, 80–250 × 7–10 μ, three- to ten-septate. Spores subhyaline to yellowish, 53–135 × 15–23 μ, subcylindrical with hemispherical or hemiellipsoidal ends, or widest somewhat below the middle and tapering moderately towards the ends; the apical portion sometimes produced into a somewhat narrowed distal prolongation, one- to eleven-septate, scarcely or not constricted at the septa. Germination is by production of germ tubes from several or all segments. To this species is tentatively ascribed a fungus collected on dead basal leaves of *Agrostis* sp., Kew Gardens, 15 June 1945. A number of species of *Helminthosporium* are known to occur on *Agrostis* but *Helminthosporium stenacrum* is characterized by and named for 'the somewhat attenuated distal prolongation characteristic of many of the spores', a character clearly displayed by several of

those figured here. As in Drechsler's experience, the conidiophores were found on dry withered leaves, not associated with any definite lesion. In this the Kew material seems to differ from *H. erythrospilum* described by Drechsler (1935) as causing a leaf spot of *Agrostis*.

Alternaria Anagallidis Raabe in *Hedwigia*, LXXVIII, 87 (1938).

Spots mainly along the edges of the leaves, rounded, brown, concentrically zoned, up to 1 cm. across; conidiophores sparse. Conidia pallid-

Fig. 22. *Helminthosporium stenacrum* Drechsl. *a*, nine conidia from dead leaves of *Agrostis* sp.; *b*, three conidiophores (× 300).

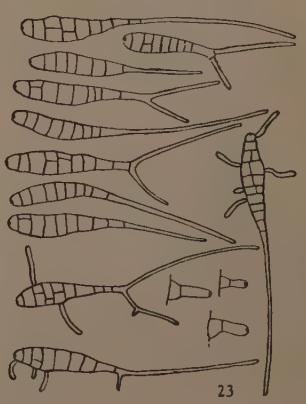


Fig. 23. *Alternaria Anagallidis* Raabe. Conidia from leaf-spots of *Anagallis arvensis* kept overnight in damp Petri dish; three germinated after 3 hours in tap water. . Conidiophores from lesions collected out-of-doors (× 200).

olivaceous, clavate, drawn out into a long beak which is sometimes forked, multiseptate, finally muriform, 95–235 μ long (including beak), 10–26 μ broad. On living leaves of *Anagallis arvensis* (scarlet pimpernel).

This fungus was very abundant in July 1945 on living leaves of scarlet pimpernel at Kew. In addition to the foliar lesions mentioned by Raabe the stems were often attacked and girdled, resulting in the death of whole branches. Conidia, rather sparsely produced on the lesions out of doors, appeared in profusion on those kept overnight on moist filter paper in a Petri dish. The conidia in the Kew material measured 100–180 × 12–15 μ.

Neergaard (1945), who obtained *Alternaria Anagallidis* on *Anagallis*

arvensis and *A. arvensis* var. *caerulea* at Copenhagen and studied it in agar culture, remarks that it resembles the forms of *Alternaria Porri* (his name for the group of allied species including *A. Solani*), but differs in the considerably shorter spore-body, in cultural characters and in host relationships. In his infection experiments the fungus attacked seedlings of *Linaria maroccana* and, to a slight extent, lettuce, onion, *Dianthus*, *Godetia*, *Nicotiana* and *Antirrhinum*.

Oidiodendron fuscum Robak in *Nyt Mag. for Naturvidenskab.* LXXI, 249 (1932).

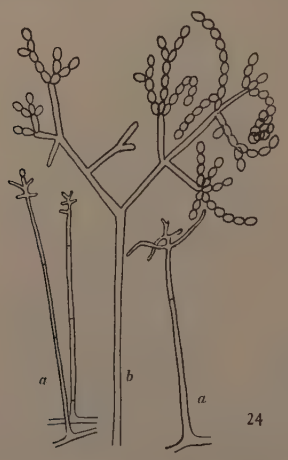
In July 1943 one of us (R. W. G. D.) collected from a prostrate log on Corstorphine Hill, Edinburgh, a patch of bark several inches long bearing a copious growth of a short grey mould which proved of much interest. In general appearance it recalled *Hormodendron cladosporioides*, now regarded as a phase of *Cladosporium herbarum*. Spores planted on 2% malt agar, however, yielded cultures quite unlike *Cladosporium* and consisting of a slow-growing grey-brown mat with a distinct tendency to become lumpy and heaped up at intervals. The under-surface of the cultures appeared deep brown and finally almost blackish brown though never quite black, due to a change in colour of the medium rather than to that of the hyphal mat. Microscopic examination revealed abundant tall brown conidiophores, much branched at the top and ultimately crowned by densely clustered chains of unicellular grey-brown conidia. The fungus is evidently a species of *Oidiodendron*, established by Robak in 1932. He described it as resembling *Hormodendron*, from which it differed in the conidiophore branches dividing into equal segments which rounded off as spores. It was said to be 'nearest to a further development of the type which is represented by the genus *Oidium* (Link) Sacc. and by species of the genus *Torula* Pers. where the spores develop in chains by the division of the ordinary hyphae'. This type of spore formation is clearly shown by cultures of the Scottish material. Robak recognized three species, *Oidiodendron nigrum*, *O. fuscum* and *O. rhodogenum*. The latter cannot be the species from Scotland as it has hyaline mycelium and usually forms a red stain in the medium or on wood pulp. Of the others, the Corstorphine fungus agrees better with *O. fuscum* which is distinguished from *O. nigrum* by its smaller spores ($1{\cdot}6$–$3{\cdot}5 \times 1{\cdot}2$–$2{\cdot}2\,\mu$ instead of $2{\cdot}2$–$5{\cdot}4 \times 2$–$3{\cdot}7\,\mu$), longer conidiophores, more clearly differentiated from the mycelium, and by the colour of the conidial layer on wood pulp, which is grey-brown to chocolate brown instead of sooty black. The diagnosis is as follows:

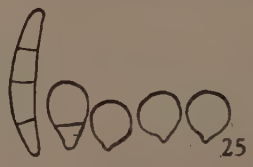
Oidiodendron fuscum Robak. Immersed hyphae 1–$1{\cdot}5\,\mu$ thick, aerial hyphae usually slightly coarser, smooth, at first pale brown, later dark brown. Conidia brownish green or dark blue-grey, occasionally hyaline, $1{\cdot}6$–$3{\cdot}6 \times 1{\cdot}2$–$2{\cdot}2\,\mu$, average $2{\cdot}4 \times 1{\cdot}7\,\mu$. Conidiophores tree-like, glabrous 60–200, seldom up to $265\,\mu$ and mostly 100–$110\,\mu$ long, main stem cylindrical, $1{\cdot}5$–$2\,\mu$ thick, unbranched for more than half its length. At the edge of the cultures single chains of slightly larger conidia may occur.

Fusarium Poae (Peck) Wollenw. apud Lewis, *Maine Agric. Expt. Stat. Bul.* 219 (1913).

Sporotrichum Poae Peck in 56*th Ann. Rept N.Y. State Museum*, 29 (1904) (for full synonymy see Wollenweber and Reinking, 1935).

A fungus isolated from pink-stained oats from an Aberdeenshire crop was determined as *Fusarium Poae* by Dr G. R. Bisby, who was familiar with the species in Canada. The same organism had been collected previously on a cock's-foot (*Dactylis glomerata*) head much damaged by insects at Corstorphine. In cultural characters it agrees with the description given by Wollenweber and Reinking: Growth cobwebby or a woolly felt, white or rosy, consisting of much-branched hyphae and conidiophores, branches alternate, dichotomous or whorled, stroma colours carmine-red-purple, ochre-yellow or violet. Conidia forming an isabelline or whitish powder over the mycelium or clustered into a sandy or mealy layer on the substrate.





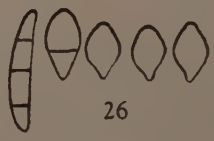
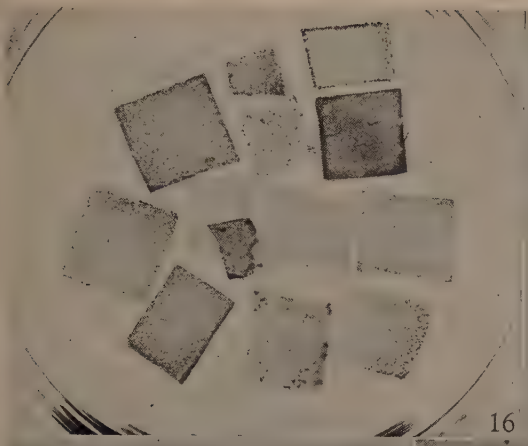
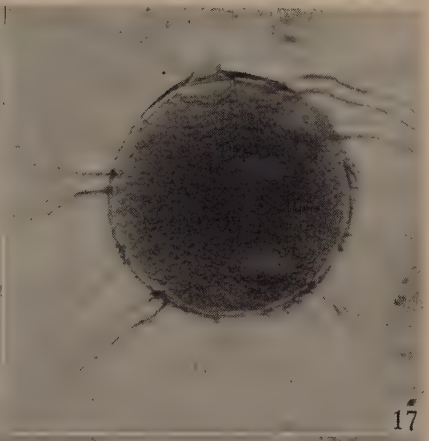


Fig. 24. *Oidiodendron fuscum* Robak, from 18 days' old culture on malt agar. *a*, three conidiophores mounted in lactophenol, spores removed, showing branching (×400); *b*, apical portion of conidiophore with spores attached (×800).
Fig. 25. Conidia of *Fusarium Poae* (Peck) Wollenw. apud Lewis (×750).
Fig. 26. Conidia of *F. citriforme* Jamalainen. (×750, reduced from Jamalainen, 1943.)

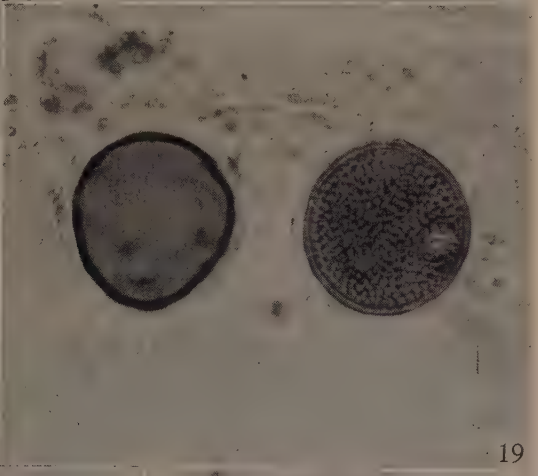
In the original description of the species by Peck, only subglobose, hyaline conidia 4–8 μ in diameter were mentioned, hence it was classed as a species of *Sporotrichum*. Similarly only *Sporotrichum*-like conidia have been seen either on the cock's-foot head or in the cultures derived from it and from the discoloured oats. Wollenweber and Reinking, however, obtained in their cultures up to 4% of conidia with one- to four-septa and consequently transferred the species to *Fusarium*. Their conidia were nearly spherical, lemon-shaped (the form with lemon-shaped conidia has been separated by Jamalainen as *F. citriforme*), or pear-shaped, non-septate, 5–12 × 3–8 μ or one-septate and 9–20 × 3·5–9 μ, occasionally longer, spindle-shaped, non-septate 10–15 × 2·5–4 μ or one-septate 12–26 × 2·7–5 μ or sickle-shaped and three-septate, 18–35 × 3·5–5 μ.

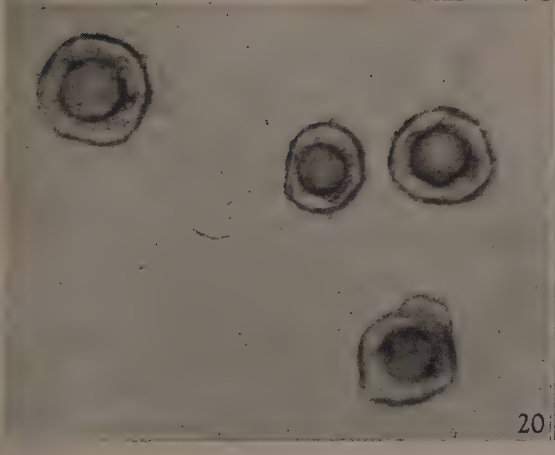
Peck's original collection was on sheaths and culms of *Poa pratensis*, but

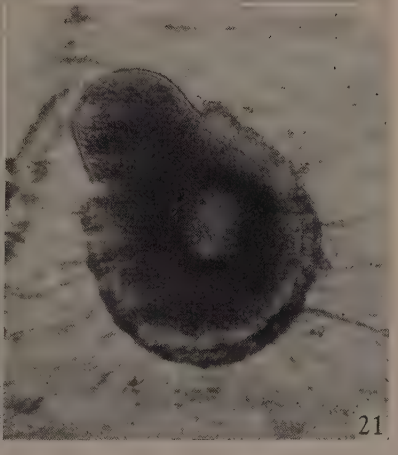

16


17

18


19


20


21

Sprague has reported this species as discolouring oat panicles in Oregon and one of Wollenweber's collections was on oat. The fungus is also regarded as the cause of bud rot of carnation and has been found associated with blighted ears of *Agrostis*, *Avena byzantina*, *Bouteloua gracilis*, *Bromus inermis*, *Phleum*, *Poa*, barley and wheat in North America and with foot-rot of rye in Italy. Other records are as a saprophyte on peas injured by foot-rot and root-rot in U.S.A., as an associate of root-rots of lucerne, *Medicago falcata* and *Melilotus* spp. in Canada, on red clover seed in U.S.S.R., on stored citrus fruit in Georgia and on ripe peaches in Italy. Its known distribution includes U.S.A., Canada, Argentina, New South Wales, U.S.S.R., Italy and Germany and Jamalainen (1943) has recently reported it from Britain. Oat seed inoculated with the culture from Aberdeenshire oats gave a full stand of healthy seedlings, so the species seems unlikely to be of economic importance as a parasite of British cereals. It should be noted, however, that most of the foreign records indicate that the part attacked in the Gramineae is the maturing panicle, usually in association with the grass mite *Pediculopsis graminum*.

Jamalainen (1943) also recorded his closely related species *Fusarium citriforme* Jamalainen 'In cariops Tritici, Britannia, Newcastle-upon-Tyne, 1935, leg. Bennet'. This species differs from *F. Poae* in its lemon-shaped one-celled conidia.

Note. Since the description of *Psilocybe cyanescens* was sent to press, our attention has been called to a figure of *Hypholoma cyanescens* R. Maire (1928) in *Bull. Soc. Mycol. Fr.* LVIII, 1942, with a redescription of the fungus by Malençon on p. 49. This North African fungus resembles ours very closely, and may prove to be the same. It differs however in certain points, notably in the more campanulate pileus (according to Malençon) and in the adnexed gills. It is noteworthy that Malençon suggests relationship to certain species of *Psilocybe*.

REFERENCES

Berkeley, M. J. & Broome, C. E. (1871). Notices of British fungi. *Ann. Mag. Nat. Hist.* (4), VII, 425–36.

Boudier, E. & Patouillard, N. (1900). Note sur deux champignons hypogés. *Bull. Soc. Mycol. Fr.* XVI, 141–6.

Bubák, Fr. & Kabát, J. E. (1904). Dritter Beitrag zur Pilzflora von Tirol. *Oesterr. bot. Zeitschr.* LIV, 181–6.

von Büren, G. (1915). Die Schweizerschen Protomycetaceen. *Beitr. Krypt. Flora der Schweiz*, V, 1.

von Büren, G. (1922). Weitere Untersuchungen über die Entwicklungsgeschichte und Biologie der Protomycetaceen. *Beitr. Krypt. Flora der Schweiz*, V, 3, 23–5.

Clements, F. E. & Shear, C. L. (1931). *The Genera of Fungi.*

Crabill, C. H. (1912). Studies on *Phyllosticta* and *Coniothyrium* occurring on apple foliage. *Ann. Rept Virginia Agric. Expt. Sta.* 1911–12, pp. 95–115.

Cunningham, G. H. (1944). The Gasteromycetes of Australia and New Zealand.

Dennis, R. W. G. (1943). A black rot of apples new to Britain. *Gard. Chron.* (3), CXIV, 221 and 223.

Drayton, F. L. (1934). The sexual mechanism of *Sclerotinia Gladioli*. *Mycologia*, XXVI, 46–72.

Drechsler, C. (1923). Some graminicolous species of *Helminthosporium*, I. *Journ. Agric. Res.* xxiv, 641–740.
Drechsler, C. (1935). A leaf spot of bent grasses caused by *Helminthosporium erythrospilum* n.sp. *Phytopathology*, xxv, 344–61.
Fischer, G. W. & Hirschhorn, E. (1945). The Ustilaginales or 'Smuts' of Washington. *Washington Agric. Expt. Sta. Bull.* 459.
Gola, G. (1930). *L'erbario micologico di P. A. Saccardo*, p. 87.
Jackson, H. S. (1935). The nuclear cycle in *Herpobasidium filicinum* with a discussion of the significance of homothallism in Basidiomycetes. *Mycologia*, xxvii, 553–72.
Jamalainen, E. A. (1943). Ueber die Fusarien Finlands, II. *Landwirtsch. Versuchsanst. Abt. f. Pflanzenkr.* 1943, pp. 1–11.
Massee, G. & Salmon, E. S. (1901). Researches on Coprophilous Fungi. *Ann. Bot.* xv, 313–57.
Neergaard, P. (1945). Danish Species of *Alternaria* and *Stemphylium*.
Petch, T. (1938). British Hypocreales. *Trans. Brit. Mycol. Soc.* xxi, 243–305.
Petch, T. (1939). Gliocladium. *Trans. Brit. Mycol. Soc.* xxii, 257–63.
Potebnia, A. (1907). Verzeichnis der in Mittel-Russland (Gouv. Kursk und Charkov) gesammelten Pilze. *Ann. Mycol.* v, 12–28.
Prillieux, E. E. & Delacroix, G. (1890). Sur le *Phoma Mali* n.sp., parasite des feuilles de Pommier. *Bull. Soc. Mycol. Fr.* vi, 180–1.
Saccardo, P. A. (1878). Fungi Veneti novi vel critici vel Mycologiae Veneti addendi, series VII. *Michelia*, i, 133–221.
Salmon, E. S. (1907). Apple leaf-spots. *Gard. Chron.* (3), xlii, 305–6.
Sampson, K. (1940). List of British Ustilaginales. *Trans. Brit. Mycol. Soc.* xxiv, 294–311.
Sheldon, J. L. (1907). The taxonomy of a leaf-spot fungus of the apple and other fruit trees. *Torreya*, vii, 142–3.
Thaxter, R. (1922). Reliquiae Farlowianae distributed from the Farlow Herbarium of Harvard University. *Mycologia*, xiv, 99–103.
Thom, C. (1930). *The Penicillia.*
Winter, G. (1873). Mycologische Notizen. *Hedwigia*, xii, 145–7.
Wollenweber, H. W. & Hochapfel, H. (1937). Beiträge zur Kenntnis parasitarer und saprophytischer Pilze. IV. *Coniothyrium* und seine Beziehung zur Fruchtfaule. *Zeitschr. f. Parasitenkunde*, ix, 600–37.

(*Accepted for publication* 23 *May* 1946)

UROMYCES STRIATUS SCHROET. ON *MEDICAGO LUPULINA* L. AND OTHER HOST PLANTS IN BRITAIN

By H. H. GLASSCOCK and W. M. WARE

A seven acres field of trefoil (*Medicago lupulina* L.) near Faversham, Kent, was visited by us on 17 October 1945. As far as could be ascertained, every plant was infected by a rust which on microscopic examination was found to agree with a description (Grove, 1913) of the uredo and teleuto stages of *Uromyces striatus* Schroet. The attack was particularly intense in large, well-defined patches where the leaves of the plants had been killed and where mature pustules were so abundant that when the plants were disturbed by walking amongst them, a cloud of spores rose in the air and a visible deposit was left on boots and clothing. These areas of very heavy infection corresponded to places where the previous wheat crop had been thinner and where the ley had 'taken' first with more numerous plants. Pustules of the fungus were present on both sides of the laminae but were far more abundant on the lower surface. They were also seen on petioles, stipules and peduncles.

No sign of the fungus could be found on an adjoining crop of lucerne (*Medicago sativa* L.), a plant which is commonly attacked by *Uromyces striatus* in the U.S.A. and in parts of Europe.

The farmer reported that a second field of twenty acres of trefoil, situated a few miles away, and grown from seed of the same delivery, was similarly infected. Damage to both crops was so severe that they were useless for forage and would, therefore, be ploughed in.

The aecidial stage of *U. striatus* is reported by Grove (1913) to occur on *Euphorbia Cyparissias* L. but to be unknown in Britain. This alternate host which is stated by Butcher (1930) to be 'a not uncommon casual weed, especially in E. England and possibly a native', was not looked for at the time of our visit.

A second outbreak of *Uromyces striatus* on trefoil, this time in the West of England, was observed by Mr L. Ogilvie and Dr Moira C. D. Munro who inform us that they found it in experimental plots at Chedzoy near Bridgwater, Somerset, on 29 November 1945.

A search was made of the literature for previous mention of *U. striatus* on trefoil and on other hosts in Britain but only two (of doubtful value) relating to trefoil were found. One of these is in the *List of Common British Plant Diseases* (all editions) compiled by the Plant Pathology Committee of the British Mycological Society; the other is that by Ainsworth (1937) who cites Grove (1913). The sole statement made by Grove (1913) which can refer to trefoil is that '*U. striatus* is found...on many species of *Medicago*, including all the British species, but I have seen no specimens on these from this country'.

Actual records of *Uromyces striatus* on other hosts (Grove, 1913) are 'uredo- and teleutospores on leaves and stems of *Trifolium minus*, Bath (Herb. Broome); King's Norton (Worcestershire). Very uncommon. July–August.... The teleutospores on *T. minus* which I have observed are more distinctly verrucose and less striated than in the figures given by Fischer, and may possibly not belong to the same species.' Ramsbottom (1914) pointed out: 'The species of *Uromyces* on *Trifolium minus* in the Broome Herbarium has been found on examination to be *U. Jaapianus* Kleb. This was previously lumped with *U. striatus*. The other record in Grove is also on *T. minus*, and is also probably *U. Jaapianus*.' It should be noted here that three of the spores figured by Grove as *U. striatus* (ex Herb. Broome) are now known to be spores of *U. Jaapianus*.

Another mention of *U. striatus* on *Trifolium minus* is made in a list, compiled by Ramsbottom (1921), of Uredineae found at Porlock during the Minehead foray of the British Mycological Society, 27 September–2 October 1920.

In connexion with this foray, Dr Alex Smith of the Plant Pathology Laboratory, Harpenden, informs us *in litt.* that a specimen exists at that laboratory originally labelled *Puccinia striatus* on *Medicago arabica*. This specimen was collected at the Minehead foray in 1920. In the absence of teleutospores (which are always uncommon on this host) identification is less certain, but the name on the label has been changed to *Uromyces striatus* because the original was presumably a *lapsus calami*. Dr Smith further informs us that because no mention is made of *Medicago arabica* as a host plant in the published list of fungi collected on the foray (Ramsbottom, 1921), he sent the specimen to the Royal Botanic Gardens, Kew, where its identity as *M. arabica* was confirmed.

Thus, resulting from the Minehead foray, there is a hitherto unrecorded specimen of *Uromyces striatus* on *Medicago arabica* in the Herbarium at Harpenden and a record (Ramsbottom, 1921) of *Uromyces striatus* on *Trifolium minus* but with no statement as to the existence of any herbarium specimen.

Uromyces striatus on *Trifolium minus* is further listed by Hadden (1920) as occurring on a lawn at Porlock. It is not known whether this record is distinct from that by Ramsbottom (1921) already cited from Porlock. We have received from Mr Hadden a specimen of the fungus which he listed in 1920. Uredospores only were present; they showed that the fungus was *Uromyces Jaapianus*. The specimen was sent to the British Museum (Natural History) and our opinion was confirmed by Dr Ramsbottom.

In a table showing characteristics of the Rusts of British herbage legumes, Sampson and Western (1941) included *U. striatus* on *Medicago maculata*. We are informed by one of these authors that this host plant was cited by them as a result of a communication from Dr Alex Smith who was referring to the specimen of *M. arabica* collected at the Minehead foray. The synonym *M. maculata* was used.

Hadden (1916) made what he considered to be the first record in Britain of *Uromyces striatus* on *Trifolium procumbens* from specimens found at Hollerday Hill and Lynbridge Lane, north Devon. A specimen of this was re-

ceived from Mr Hadden. Teleutospores were absent but the uredospores showed that the fungus was *Uromyces Jaapianus*. This identification was also confirmed at the British Museum (Natural History).

Thus, in the literature to which reference has been made, it appears that existing records of *U. striatus* stand for only one host in Britain, namely *Medicago arabica*. From the fact that every British specimen of *Uromyces striatus* on *Trifolium* spp. examined by us has proved to be *Uromyces Jaapianus*, it would seem that all published records of *U. striatus* occurring on *Trifolium* spp. will need confirmation.

Klebahn (1913), under his new species *Uromyces Jaapianus*, included (pp. 239–40) only the one specimen on *Trifolium minus* collected by Jaap. On pp. 244–5 under *Uromyces striatus* he still included as hosts *Trifolium agrarium*, *T. minus*, *T. procumbens* and *T. arvense*, but it is not known whether he had examined critically the exsiccati he enumerated.

Uromyces striatus is now recorded for the first time on a crop plant, *Medicago lupulina*, in Britain. Specimens have been sent to the Herbaria at Kew and the British Museum.

(*Accepted for publication* 29 *May* 1946)

REFERENCES

Ainsworth, G. C. (1937). *The Plant Diseases of Great Britain*, p. 27. London.
Butcher, R. W. (1930). *Further Illustrations of British Plants*, p. 307. 1st ed. Ashford.
Grove, W. B. (1913). *The British Rust Fungi*, p. 93. Cambridge.
Hadden, N. G. (1916). Uredinales of north Devon. *J. Bot., Lond.*, liv, 52–4.
Hadden, N. G. (1920). The Uredineae of west Somerset. *J. Bot., Lond.*, lviii, 37–9.
Klebahn, H. (1913). *Kryptogamenflora der Mark Brandenburg*, v*a*. Pilze.
Ramsbottom, J. (1914). Notes on nomenclature of some rusts. *Trans. Brit. Myc. Soc.* iv, 332.
Ramsbottom, J. (1921). List of species gathered during the Minehead Foray. *Trans. Brit. Myc. Soc.* vii, 7.
Sampson, K. & Western, J. H. (1941). *Diseases of British Grasses and Herbage Legumes*, p. 61. Cambridge.

PREDACIOUS FUNGI IN BRITAIN

By C. L. DUDDINGTON, M.A., *Biological Laboratories, The Polytechnic, Regent Street, London*

In 1940, I recorded the occurrence of four species of Zoopagaceae in this country, namely, *Cochlonema verrucosum* Drechsl., *C. dolichosporum* Drechsl., *Stylopage hadra* Drechsl., and *S. haploe* Drechsl. All four species were obtained from samples of leaf-mould from Guiting Wood, near Winchcombe, Gloucestershire.

Since that time, further observations have been made, and these have yielded the following first records for this country:

(1) *Cystopage lateralis* Drechsl., a member of the Zoopagaceae, capturing and consuming nematodes in a sample from an old manure heap at Coppice Corner Farm, Lowsonford, Warwickshire.

(2) *Protascus subuliformis* Zopf, attacking nematodes in the same sample which yielded *Cystopage lateralis*.

(3) *Stylopage* species, capturing and consuming small amoebae. The mycelium consisted of delicate hyphae, 0·7–1·2 μ in diameter, bearing ovoid conidia on slender conidiophores which, by continued growth, might bear more than one conidium. The amoebae were held on the mycelium, apparently by some sticky substance, and their contents were absorbed by the fine, branched haustoria. The species could not be determined with certainty because of the lack of mature material, but it seemed to be near to *S. lepte* Drechsl. It occurred on rotting plant debris from the margin of a pond at Bushwood Farm, Lowsonford, Warwickshire (Map reference, O.S. 1/63360, sheet 72, 637890).

(4) *Meria coniospora* Drechsl., attacking nematodes in a sample of well-rotted cow dung from a manure heap at Coppice Corner Farm, Lowsonford.

(5) *Harposporium anguillulae* Lohde., parasitic on nematodes, has been observed twice; in leaf mould from Brick Kiln Hole Wood, Solihull, Warwickshire, and in old cow dung from Coppice Corner Farm, Lowsonford.

(6) *Dactylella bembicoides* Drechsl., capturing nematodes by means of constricting rings, in old cow dung from Coppice Corner Farm, Lowsonford.

Work is in progress on these and other forms, and it is proposed at a later date to give further details of the systematics and biology of British predacious fungi.

REFERENCE

Duddington, C. L. (1940). Predacious fungi from Cotswold leaf mould. *Nature, Lond.*, cxlv, 150–1.

(*Accepted for publication* 31 *May* 1946)

A MULTIPLE-POINT INOCULATING NEEDLE FOR AGAR PLATES

By S. D. GARRETT, *Rothamsted Experimental Station, Harpenden*

(With 1 Text-figure)

Recent work in this laboratory has required the inoculation of agar plates at ten points with agar mycelial disks of a non-sporing fungus. As at least a hundred plates had to be inoculated at one time, ten separate transfers of agar disks to each inoculant plate would have entailed not only waste of time, but also considerable risk of contamination, in opening and closing the Petri dishes so many times. A ten-point inoculating needle was therefore devised and constructed, as follows. Ten 15 cm. lengths of steel

Fig. 1

wire, No. 20 gauge, were cut, and one end of each was hammered out flat on an anvil. Each length of wire was bent through an angle of 60° at a distance of 6 cm. from the flattened end, and then through an angle of 90° in the reverse direction at a distance of 10 cm. from the same end, both angles being in the plane of the flattened tip of the wire. The ten lengths of bent wire were then clamped to a wooden holder, in which grooves had been cut, to give the finished instrument (see Fig. 1).

After sterilizing the ten points in a flame, the instrument was used as follows. The inoculum plate, in which agar disks had been cut with a

sterile cork borer, was held upside down without its lid above eye level, and an agar disk removed on the spatulate end of one of the ten 'points'. The instrument was given a slight twist and a second disk was removed on the next point, and so on until all ten points were carrying disks. The inoculum plate was then replaced in its lid, and the inoculant plate was picked up and held in a similar position, while the ten inoculum disks were transferred one by one to their correct positions, previously marked out by means of a stencil on the bottom of the dish.

The number of plates thus inoculated which have to be discarded owing to contaminations does not much exceed that lost when plates are inoculated at a single central point. In the first trial of this technique, the inoculation of sixty-five plates took four hours, and six plates each developed a single contaminant colony, but of these only two plates had to be discarded as useless.

(*Accepted for publication* 31 *May* 1946)

A SUBSTANCE CAUSING ABNORMAL DEVELOPMENT OF FUNGAL HYPHAE PRODUCED BY *PENICILLIUM JANCZEWSKII* ZAL.

I. BIOLOGICAL ASSAY, PRODUCTION AND ISOLATION OF 'CURLING FACTOR'

By P. W. BRIAN, P. J. CURTIS and H. G. HEMMING

Hawthorndale Laboratories, Jealott's Hill Research Station

(With 2 Text-figures)

In the course of an investigation of the toxicity of Wareham Heath soil (Brian, Hemming & McGowan, 1945) it was noted that the mould flora was composed almost entirely of species of *Penicillium*, as had been previously recorded by Neilson-Jones (1941). It was further shown that these species, with but a few exceptions, fall into three species or groups:

(*a*) *P. Janczewskii* Zal.

(*b*) *P. Jenseni* Zal.

(*c*) A group of strains falling into the *P. nigricans-Janczewskii* series of Thom (1930) but distinct from those identified as *P. Janczewskii*.

P. Jenseni was shown to produce the antibiotic substance known as gliotoxin. The strains in group (*c*) produced an antibiotic which had not at that time been isolated and the strains of *P. Janczewskii* produced in liquid culture-media a substance having a peculiar distorting effect on the germ tubes of *Botrytis Allii*. The present communication is concerned with more detailed investigations of the production and properties of this latter substance.

Cultural characteristics of *Penicillium Janczewskii*

Colonies on Czapek-Dox agar develop a closely meshed felt almost velvety in appearance. The colonies show radial folds and a convoluted and raised centre, with a white margin about 1 mm. wide. Conidial areas are greenish-grey, becoming almost black and the reverse yellow, ochraceous brown, or purplish brown. The odour is strong and reminiscent of *P. expansum*. The penicillus is of the divaricate type characteristic of the *Lanata-Divaricata*, showing a main axis with divergent branches. The sterigmata are 7 μ long, the conidia spherical and roughened, 2·5–3 μ in diameter. Four strains could be distinguished, varying slightly in the colour of the colony reverse, but all the work described below has been carried out on one strain only (no. 250). These strains were kindly examined by Dr K. B. Raper (United States Dept. of Agriculture) who agrees that they fall into the *nigricans-Janczewskii* series, but makes the point that it is questionable if one can differentiate satisfactorily between the species *nigricans* and the species *Janczewskii*.

Nutrient requirements of *Penicillium Janczewskii*

P. Janczewskii grows well on the ordinary synthetic media. It can utilize either ammonium salts or nitrates as a nitrogen course; growth is more rapid with an ammonium nitrogen source. A wide variety of substances can be utilized as a source of carbon. Only a limited range of sugars have been tested, but growth is good, in a medium of the Czapek-Dox type, with dextrose, sucrose, maltose, lactose, galactose, dextrin and starch. Growth is also good where glycerol or succinic acid are used as carbon sources. Less suitable, but utilizable by the mould, are fumaric acid, malic acid, lactic acid, acetic acid, citric acid, aconitic acid and malonic acid. The mould is not able to utilize racemic acid, mesotartaric acid, tartaric acid, glycollic acid, meconic acid, maleic acid, diethylene glycol, oxalic acid or itaconic acid. The failure to utilize tartaric acid is unusual.

Preliminary observations on antibiotic activity

(1) *Antagonism in culture.* Plates of Lemco-peptone-glucose agar (Lab-Lemco 3 g., peptone (Evans') 10 g., glucose 10 g., sodium chloride 5 g., agar 20 g., water 1000 ml.) were inoculated with a transverse streak of spores of *P. Janczewskii* and incubated for four days at 25° C. when a vigorous colony had been produced. Cell suspensions of three bacteria (*Staphylococcus aureus*, *Salmonella typhi*, *Escherichia coli*) and one pathogenic fungus (*Endomycopsis albicans*) were then streaked at right angles to the mould colony, starting at its edge. The plates were then incubated for a further two days at 37° C. No inhibition of the growth of the test organisms was observed. With moulds producing antibiotics (e.g. *P. Jenseni* mentioned above) a marked zone of inhibition near the mould colony is usually seen.

(2) *Cultures on liquid media.* The following five media have been used:

Weindling (dextrose 25·0 g., ammonium tartrate 2·0 g., potassium dihydrogen phosphate 1·0 g., magnesium sulphate, hydrated 1·0 g., minor element concentrate 1 ml., water 1000 ml.; adjusted to *p*H 3·5 with hydrochloric acid).

Czapek-Dox (dextrose 50·0 g., sodium nitrate 2·0 g., potassium dihydrogen phosphate 1·0 g., potassium chloride 0·5 g., magnesium sulphate, hydrated 0·5 g., ferrous sulphate 0·01 g., water 1000 ml.).

Raulin-Thom (dextrose 50·0 g., tartaric acid 2·66 g., ammonium tartrate 2·66 g., diammonium hydrogen phosphate 0·4 g., potassium carbonate 0·4 g., magnesium carbonate 0·27 g., ammonium sulphate 0·17 g., zinc sulphate, hydrated 0·05 g., ferrous sulphate 0·05 g., water 1000 ml.).

Cornsteep (crude glucose 20·0 g., lactose 20·0 g., sodium nitrate 3·0 g., potassium dihydrogen phosphate 0·5 g., magnesium sulphate, hydrated 0·25 g., ferrous sulphate, hydrated 0·01 g., zinc sulphate, hydrated 0·002 g., cornsteep liquor 80 ml., water 920 ml.).

Lemco-dextrose broth (peptone (Evans') 10 g., Lab-Lemco 3 g., dextrose 10 g., sodium chloride 5 g., water 100 ml.).

The minor element concentrate has the following composition: ferrous

sulphate (hydrated), 0·1 g.; copper sulphate (hydrated), 0·015 g.; zinc sulphate (hydrated), 0·1 g.; manganese sulphate (hydrated), 0·01 g.; potassium molybdate, 0·01 g.; distilled water, 100 ml.

The media were disposed in 30 ml. lots in 100 ml. Pyrex flasks, autoclaved, inoculated and incubated at 25° C. Five flasks of each medium were withdrawn at random and bulked after six, eight, ten and twelve days' growth and the filtered liquid medium assayed for antibiotic activity. The assays used were serial dilution tests with two bacteria (*Staphylococcus aureus* and *Salmonella typhi*), carried out in the conventional manner, and a serial dilution spore germination test using conidia of *Botrytis Allii* Munn. The technique of this spore germination assay has been previously described by Brian and Hemming (1945). Results of an experiment with *Penicillium Janczewskii* (no. 250) are shown in Table 1. The data given show the greatest dilutions inhibiting germination of *Botrytis Allii* conidia (i.e. B.A. units/ml.) or growth of *Staphylococcus aureus* (i.e. *Staph.* units/ml.) or *Salmonella typhi* (i.e. *Salmonella* units/ml.). Experiments with other strains of *Penicillium Janczewskii* gave similar results.

Table 1. *Fungistatic and antibacterial activity of culture filtrates from* Penicillium Janczewskii *grown at* 25° C. *on five media*

Activity	Days' growth	Weindling	Raulin-Thom	Czapek-Dox	Cornsteep	Lemco-dextrose
B.A. units/ml.	6	4	8	2	—	—
	8	4	4	4	—	—
	10	4	—	2	—	—
	12	—	—	—	—	—
Staph. units/ml.	6	4	4	4	—	—
	8	2	2	2	—	—
	10	2	2	2	—	—
	12	4	2	4	—	—
Salmonella units/ml.	6	2	2	2	—	—
	8	2	2	2	—	—
	10	2	2	2	—	—
	12	2	2	—	—	—

The antibiotic activity of the culture filtrates was relatively small throughout. The antibacterial effects of culture filtrates from Weindling, Raulin-Thom and Czapek-Dox media may probably be attributed to toxic effects of the media themselves, including the effects of acidity. The antifungal activity, though slight, is probably real. The relatively low level of antifungal activity is better appreciated when it is recalled that culture filtrates from appropriate strains of *Trichoderma viride* may reach an activity of 16,384 B.A. units/ml. (Brian, Curtis, Hemming & McGowan, 1946). In spite of the low level of fungistatic activity, as expressed in prevention of germination of conidia of *Botrytis Allii*, the culture filtrates had a marked effect on the growth form of *B. Allii*, on some media even in dilutions of 1 in 512. This took the form of stunting, distortion and increased branching in the lower dilutions of culture filtrate and a 'waving' of the hyphae in the higher dilutions. This was most marked, on this occasion, in culture filtrates from Cornsteep and Lemco-dextrose media, slightly less marked in culture filtrates from Czapek-Dox but only visible in the lower dilutions (1 in 8 and 1 in 4) of Weindling and Raulin-Thom medium.

Penicillium Janczewskii was shown by this experiment to produce a substance, conveniently designated 'curling factor', causing abnormal development of the young hyphae of *Botrytis Allii* in low concentrations. This remarkable effect, unique in our experience of culture filtrates from over two hundred moulds, appeared to be worthy of further investigation, aimed particularly at isolation of 'curling factor' in pure form. This necessitated the development of some form of quantitative assay; the procedure adopted is described in the following section.

Bio-assay of 'curling factor'

Fig. 1 shows the appearance of *Botrytis Allii* conidia after incubation for seventeen hours at 25° C. in serial dilutions of a *Penicillium Janczewskii* (No. 250) culture filtrate after 14 days' growth on Czapek-Dox medium (10% glucose supplemented by 0·1% peptone). The dilutions were made in half-strength Weindling solution in the manner described by Brian & Hemming (1945). This example may be regarded as typical.

It will be seen that the effect of the 'curling factor' produced by *Penicillium Janczewskii* is to cause (*a*) stunting of the germ-tubes, (*b*) distortion and excessive branching of the hyphae, (*c*) waving of the hyphae produced. Stunting, distortion and excessive branching of the hyphae are seen most noticeably in the first dilutions of the culture filtrate, and waving of the hyphae in the greatest dilutions. It must be emphasized that, in these concentrations of 'curling factor', percentage germination is not affected. Even where there is most marked stunting (Fig. 1 A) germ-tubes were first produced and germination reached 100% at the same time as the controls (Fig. 1 I), that is in about seven hours. The effect of 'curling factor' in such concentrations is to prevent further development after the germ tubes have been formed. It is typical of the stunted hyphae (Fig. 1 A–F) that the diameter of the hyphae is much greater than normal and that the ends of the hyphae are frequently swollen or spatulate. The distortion and excessive branching, typical examples of which are seen in Fig. 1 E and F, are associated with stunting. It appears that hyphal branching is not inhibited, or is even stimulated, but that hyphal elongation is greatly retarded. In addition, the hyphae appear to lose their sense of direction and, instead of progressing away from the point of origin, tend frequently to turn back again. The waving seen in the greater dilutions (Fig. 1 G and H) is quite characteristic, but as the culture filtrate is diluted still further growth passes imperceptibly to the normal (Fig. 1 I).

After consideration of the gradual change in the effects on spore germination produced by 'curling factor' as dilution proceeds, it will be appreciated that the development of a satisfactory quantitative assay is not easy. In the case of a spore-germination test, it is relatively easy to make a distinction between germinated spores and those that have not germinated. Here the procedure adopted has been to determine (*a*) the greatest dilution at which obvious stunting has been caused (e.g. in Fig. 1F but not G) and (*b*) the greatest dilution at which waving of the hyphae is produced. Both of these points have to be assessed arbitrarily and the result is necessarily over-dependent on subjective influences. Nevertheless it has been possible,

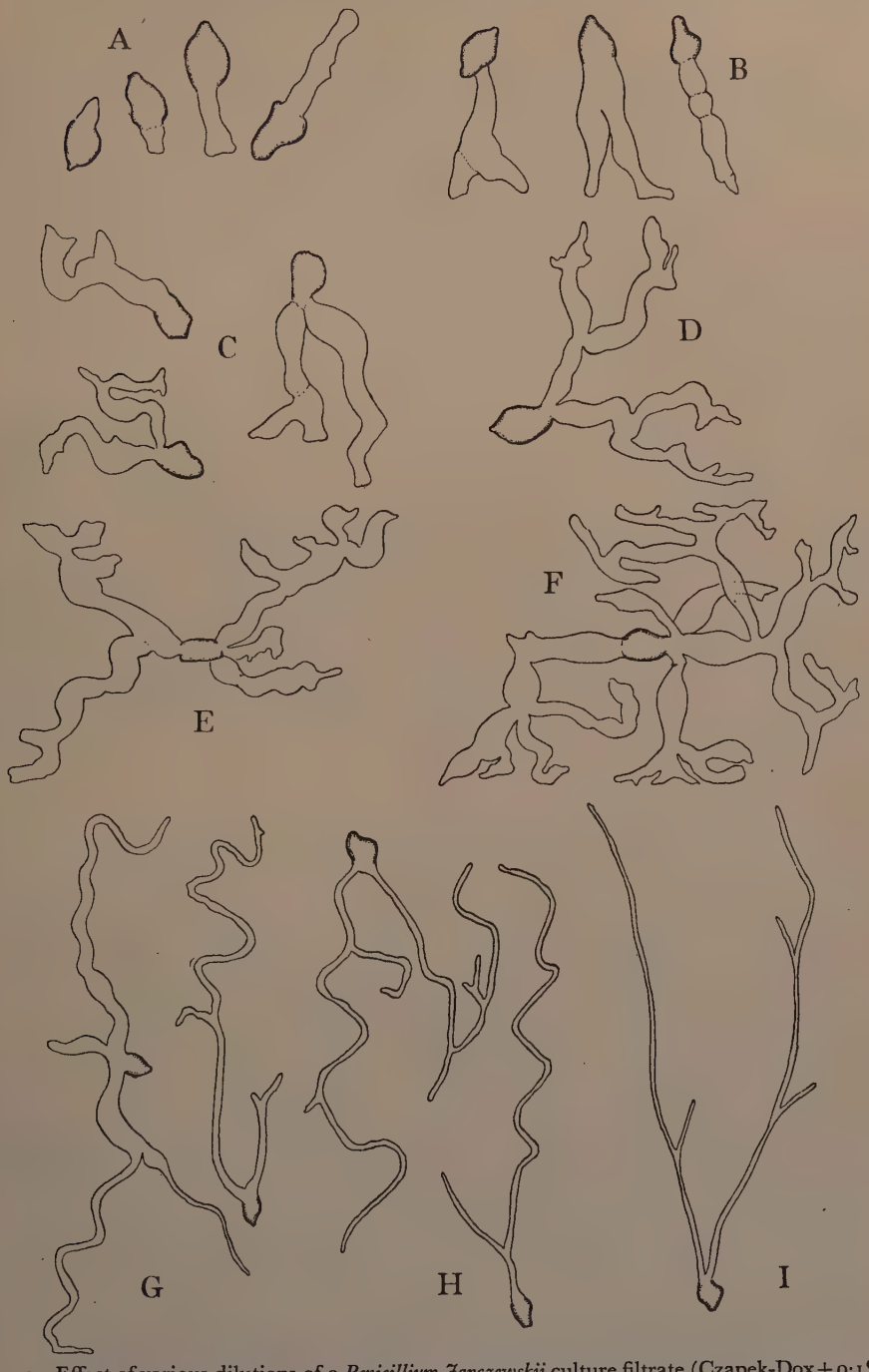


Fig. 1. Effect of various dilutions of a *Penicillium Janczewskii* culture filtrate (Czapek-Dox + 0·1% peptone) on germination of *Botrytis Allii*. Drawings made after seventeen hours' germination at 25° C. Dilutions: A, 1 in 4; B, 1 in 8; C, 1 in 16; D, 1 in 32; E, 1 in 64; F, 1 in 128; G, 1 in 512; H, 1 in 2048; I, control. (A–F × 1·5: G × 1.)

on the basis of observations of this kind, to follow the production of 'curling factor' and to develop means of extraction and purification. Results are presented as in Table 2, where, for instance, $S_{32}C_{1024}$ indicates that stunting was clearly observed in dilutions down to 1 in 32, and waving of hyphae down to a dilution of 1 in 1024.

Relation of medium composition to production of 'curling factor'

Nitrogen source

As has already been stated (see discussion of Table 1), Czapek-Dox appeared to be a more favourable medium for production of 'curling factor' than Raulin-Thom in preliminary experiments, and the cornsteep medium better than Czapek-Dox. Further experiments indicated that cornsteep was not significantly superior to Czapek-Dox but the superiority of Czapek-Dox over Raulin-Thom was confirmed. This superiority is well demonstrated by the data presented in Table 4. The main difference between these two synthetic media lies in the nitrogen source, being in the form of sodium nitrate in Czapek-Dox and various ammonium salts in Raulin-Thom. The relation between nitrogen source and 'curling factor' production was therefore studied in greater detail.

The media used were somewhat similar to Czapek-Dox in composition, and nitrogen was supplied as potassium nitrate, ammonium nitrate, ammonium tartrate or Bacto-tryptone in amounts giving equal nitrogen concentrations. The detailed compositions of the media used are given below:

Medium N:

Potassium nitrate	2·3 g.
Potassium dihydrogen phosphate	1·0 g.
Magnesium sulphate, hydrated	0·5 g.
Dextrose	75·0 g.
Minor element concentrate	1 ml.
Distilled water	1000 ml.

Medium AN: as *N* but with potassium nitrate replaced by 0·95 g. ammonium nitrate.

Medium A: as *N* but with potassium nitrate replaced by 2·2 g. ammonium tartrate.

Medium T: as *N* but with potassium nitrate replaced by 2·5 g. Bacto-tryptone.

Medium NP: as *N* but with potassium dihydrogen phosphate increased to 10·0 g./l.

Medium ANP: as *AN* but with potassium dihydrogen phosphate increased to 10·0 g./l.

Medium AP: as *A* but with potassium dihydrogen phosphate increased to 10·0 g./l.

Medium TP: as *T* but with potassium dihydrogen phosphate increased to 10·0 g./l.

These media were all adjusted (when necessary, viz. *N*, *AN*, *A*, *T*, *AP* and *TP*) to *p*H 4·4 with *N*/1 hydrochloric acid before autoclaving; 500 ml. lots of each were disposed in 'Glaxo' culture vessels with six replicates of each, inoculated with *Penicillium Janczewskii* spores and incubated at 25° C. Periodically samples were taken from each culture vessel, samples from similar media bulked, assayed for 'curling factor' activity and the *p*H determined electrometrically. Results are shown in Table 2.

There were minor growth differences on these media. After three days' growth all were pure white, with no visible sporulation, a completely confluent mycelial felt having been formed on all media except *N* and *NP*, where the growth still consisted of numerous mycelial 'islands' which had not yet fused. By visual observation the order of growth vigour was

Table 2. *Development of 'curling factor' activity in cultures of* Penicillium Janczewskii *on media with various sources of nitrogen*

Days' growth	Medium							
	N	*NP*	*A*	*AP*	*AN*	*ANP*	*T*	*TP*
	Activity by bio-assay							
3	$So Co$	$So Co$	$S_4 Co$	$So Co$	$So Co$	$So Co$	$S_4 Co$	$So Co$
4	$So C_8$	$So C_{32}$	$S_{32} Co$	$S_{32} Co$	$S_4 Co$	$S_4 Co$	$S_8 Co$	$S_4 Co$
5	$S_{16} C_{64}$	$S_8 C_{128}$	$S_{16} Co$	$S_{32} Co$	$S_{16} Co$	$S_8 Co$	$S_{16} Co$	$S_{16} Co$
6	$S_{64} C_{512}$	$S_{32} C_{1024}$	$S_{16} Co$	$S_{32} Co$	$S_{32} Co$	$S_{32} Co$	$S_{64} Co$	$S_{32} C_{64}$
7	$S_{32} C_{1024}$	$S_{32} C_{1024}$	$S_{32} Co$	$S_{16} Co$	$S_{64} Co$	$S_{32} Co$	$S_{64} Co$	$S_8 C_{128}$
10	$S_{32} C_{1024}$	$S_{32} C_{1024}$	$S_{32} Co$	$S_{32} Co$	$S_{32} Co$	$S_{32} Co$	$S_{32} Co$	$S_{16} C_{64}$
13	$S_{32} C_{8192}$	$S_{32} C_{512}$	$S_{16} Co$	$S_{32} Co$	$S_{32} Co$	$S_{32} Co$	$S_{64} C_{64}$	$S_{32} C_{512}$
17	$S_{32} C_{4096}$	$S_{64} C_{512}$	$S_{16} Co$	$S_{16} C_{128}$	$S_{16} C_{64}$	$S_{16} C_{256}$	$S_{64} C_{512}$	$S_{16} C_{256}$
24	$S_8 C_{2048}$	$S_8 C_{256}$	$S_8 C_{128}$	$S_{16} C_{512}$	$S_8 C_{256}$	$S_4 C_{128}$	$S_8 C_{512}$	$S_{32} C_{512}$
	Drift of *p*H							
3	4·9	5·1	2·6	2·9	3·1	3·7	3·9	4·0
4	4·2	4·0	2·6	3·0	4·3	4·1	4·1	4·3
5	3·8	3·7	2·6	2·9	4·3	4·2	5·1	4·4
6	3·6	3·6	2·6	2·9	4·6	4·2	4·6	4·2
7	3·4	3·5	2·5	2·9	4·6	4·3	4·7	4·3
10	3·5	3·5	2·6	3·0	4·4	4·1	4·5	4·2
13	3·6	3·7	2·7	3·1	4·3	4·3	4·5	4·2
17	3·7	3·7	2·7	3·1	4·4	4·2	4·5	4·3
24	3·8	3·8	2·8	3·1	4·1	4·0	4·2	4·2

TP > *T* > *ANP* > *AN* > *NP* > *N*. Sporulation started within the next few days appearing earliest on *T* and *TP* and last on *N* and *NP*. After six days' growth most felts were sporulating vigorously, the conidial masses being a dull sage green in colour. Thereafter the conidial masses became progressively more grey and at the close of the experiment little green colour remained.

The assay figures in Table 2 show clearly that potassium nitrate nitrogen was best for development of 'curling factor' activity, since activity developed rapidly and a higher peak assay was reached than on other media. The higher phosphate level led to an earlier development of activity but a lower peak level of activity. The next best medium was that with tryptone nitrogen and high phosphate level (*TP*) followed by the corresponding medium with low phosphate. These were much inferior to potassium nitrate media. The media with nitrogen presented as ammonium nitrate

or tartrate showed no development of typical 'curling factor' activity until after seventeen days' incubation, as compared with only four days with nitrate nitrogen. The ammonium nitrogen cultures did show signs of early production of some inhibiting factor as indicated in Table 2 by recording 'stunting' at several dilutions without 'curling'.

After initial fluctuations a steady *p*H was maintained on all media. This was very low on the ammonium tartrate media and almost as low on the nitrate media, suggesting production of an organic acid by the mould.

Organic supplements

In some earlier experiments it was found that addition of 0·1 % peptone or 0·01 % yeast extract to Czapek-Dox medium stimulated the development of 'curling factor' activity. Further detailed investigation failed to confirm that any consistent benefit could be obtained from such additions, or from addition of various vitamins (aneurin hydrochloride, nicotinic acid, riboflavin or pyridoxin) or amino-acids (*dl*-alanine, glycine, *l*-tryptophane, *β*-phenylalanine, *l*-tyrosine, *p*-aminobenzoic acid or glutamic acid). Addition of peptone, or yeast extract, does sometimes lead to a more rapid establishment of a confluent mycelial felt and in production experiments (see next section) 0·1 % peptone was added to all media as a routine measure.

Isolation of 'curling factor'

'Curling factor' is thermostable; it was demonstrated that culture filtrates showing 'curling factor' activity could be boiled for thirty minutes or autoclaved for twenty minutes without loss of activity. It was even found possible to evaporate culture filtrates to dryness in an open dish without appreciable loss in activity, but the dark brown gum, containing caramelized sugars, produced in this manner did not seem a suitable starting point for fractionation and other methods of concentration of the 'curling factor' were investigated.

Preliminary experiments indicated that the 'curling factor' could be removed from active culture filtrates by extraction with chloroform, *n*-butyl alcohol or ether, or by treatment with activated charcoal. Extraction of culture filtrates with ether or *n*-butyl alcohol has its disadvantages; if ether is used much solvent is lost, owing to the water solubility of ether and *n*-butyl alcohol extracts more coloured material from the medium than the other solvents. Accordingly further work was concentrated upon methods of extraction utilizing chloroform or activated charcoal.

The results of an experiment on methods of extraction are shown in Table 3. Here *Penicillium Janczewskii* was grown at 25° C. on 500 ml. lots of medium in flat earthenware vessels of the type used for production of penicillin by Abraham *et al.* (1941). The media used were Raulin-Thom (5 % dextrose) and Czapek-Dox (5% dextrose), both supplemented with 0·1 % peptone. Samples were taken after twelve days' growth. The extraction methods examined were (*a*) chloroform extraction of the culture filtrate, and (*b*) treatment of the culture filtrate with activated charcoal

followed by elution of the charcoal with chloroform or ether. Each of these methods is shown to be effective, whether judged by removal of 'curling factor' activity from the culture filtrate or by the activity of the material extracted. As a result of this and other similar experiments it was decided to concentrate in further work on chloroform extraction of culture filtrates.

After sixteen days' growth the cultures used for the extraction experiment just described were harvested and the culture filtrates extracted with chloroform. On evaporation of the chloroform at reduced pressure a yellowish gum with some crystalline material was obtained from the Raulin-Thom cultures, and a reddish-orange gum from the Czapek-Dox cultures. These gums were taken up in a little hot ethyl alcohol; on cooling pale straw-coloured crystals separated from the Raulin-Thom material and a reddish, largely amorphous powder from the Czapek-Dox material.

Table 3. *Activity by bio-assay of fractions obtained from* Penicillium Janczewskii *culture filtrates by various extraction procedures*

Fraction assayed	Medium: Czapek-Dox + peptone	Medium: Raulin-Thom + peptone
1. Untreated culture filtrate	$S_{16}C_{512}$	$S_{32}C_{256}$
2. Culture filtrate after chloroform extraction	So Co	So Co
3. Chloroform extract made up to original volume	$S_{16}C_{512}$	$S_{16}C_{128}$
4. Culture filtrate after treatment with charcoal	So Co	So Co
5. Chloroform eluate from charcoal made up to original volume	$S_{16}C_{512}$	S_8C_{128}
6. Ether eluate from charcoal made up to original volume	S_8C_{512}	S_8C_{64}

The yield of the former was 23 mg./l. of culture filtrate, of the latter 81 mg./l. Both these materials, when dissolved in water, showed by assay curling activity at a minimum concentration of 0·8 μg./ml. Preparations obtained by this process are described below as crude 'curling factor'.

Table 4 shows data for a number of batches of cultures run for production purposes in earthenware culture vessels. Four general conclusions may be drawn from these results:

(*a*) Czapek-Dox is a more favourable medium for production of 'curling factor' than Raulin-Thom; this is in general agreement with the results presented in Table 2, which indicated that nitrate nitrogen was more favourable than ammonia nitrogen for production of 'curling factor'.

(*b*) Increase in the sugar content of Czapek-Dox from 5·0% to 7·5% has resulted in increased yields of crude 'curling factor'. The colour of the product is also considerably improved; in these high glucose batches the crude 'curling factor' extracted was of a relatively high degree of purity.

(*c*) Increase in the sugar content of Czapek-Dox to 7·5% or higher markedly affects the *p*H drift; the medium maintains an almost steady *p*H with 7·5% glucose, as compared with a steady upward drift with 5·0% glucose. With 12·5% glucose the medium actually becomes more acid. These results like those from Table 2, previously discussed, suggest that in

high glucose media *Penicillium Janczewskii* produces considerable quantities of an organic acid. The effect of increasing glucose on *p*H may be responsible for the increased quantity and purity of yields of 'curling factor'.

(*d*) The activity of the culture filtrate as determined by bio-assay roughly follows the yields of crude 'curling factor'.

Effect of pure 'curling factor' on *Botrytis Allii*

A sample of pure 'curling factor', prepared from the crude product by recrystallization from a mixture of equal quantities of diethylene dioxide

Table 4. *Production of 'curling factor': yield data*

Batch no.	Medium	Glucose %	Period of incubation (days)	*p*H of culture filtrate	Assay of culture filtrate	Yield of crude 'curling factor' (mg./l.)	Appearance of crude product
42	RT	5·0	16	—	$S_{16}C_{128}$	23	Yellow-brown crystals
56	RT*	5·0	18	5·0	SoCo	Neg.	Grey powder
111A	RT	5·0	14	7·2	S_8C_{64}	Neg.	Grey powder
117	RT	5·0	12	4·1	$S_{16}C_{256}$	23	Yellow-brown crystals
121	RT	5·0	15	3·5	$S_{16}C_{128}$	9	Yellow-brown crystals
149C	RT	10·0	17	2·9	$S_{16}C_{256}$	37	Pale brown crystals
47	CD	5·0	15	7·3	S_8C_{512}	81	Reddish powder
57	CD*	5·0	18	7·3	S_4C_{256}	24	Brown crystals
111B	CD	5·0	14	7·9	S_4C_{64}	8	Brown powder
118	CD	5·0	16	7·2	$S_{16}C_{128}$	48	Yellow-brown crystals
134A	CD	7·5	16	4·8	$S_{32}C_{512}$	155	Yellow crystals
134B	CD	7·5	20	6·0	$S_{16}C_{512}$	82	Pale brown crystals
142A	CD	7·5	14	3·6	$S_{128}C_{2048}$	151	Yellow crystals
149A	CD	7·5	12	3·8	$S_{512}C_{2048}$	166	Pale yellow crystals
154A	CD*	7·5	18	4·2	$S_{128}C_{512}$	124	Brownish yellow crystals
154C	CD	7·5	14	3·6	$S_{32}C_{1024}$	65	Brownish yellow crystals
149B	CD	10·0	14	3·6	$S_{128}C_{4096}$	158	Yellow crystals
142B	CD	12·5	22	3·5	$S_{32}C_{512}$	59	Yellow crystals
154B	CD	12·5	18	3·5	$S_{32}C_{1024}$	51	Pale brown crystals

* 1000 ml. medium per culture vessel (all others 500 ml. per vessel).
RT = Raulin Thom + peptone. CD = Czapek-Dox + peptone. Neg. = negligible yield.

(dioxan) and water, as recommended by McGowan (1946), was dissolved in half strength Weindling solution and assayed by the *Botrytis Allii* spore germination technique already described. The effect produced was similar to that produced by active *Penicillium Janczewskii* culture filtrates, as may be seen by comparison of Fig. 2 (dilutions of pure 'curling factor') with Fig. 1 (dilutions of an active culture filtrate). From Fig. 2 it is apparent that concentrations of the order of 2·5 μg./ml. of 'curling factor' produced marked stunting and distortion of germ tubes and that the characteristic 'waving' of hyphae is clearly visible in concentrations of 0·16 μg./ml. It was not visible at 0·08 μg./ml.

These observations may be used to check the correlation between bio-assay of culture filtrates and yield of 'curling factor'. In Table 5 the yields from the batches dealt with previously in Table 4 are compared with an 'expected yield', calculated by assuming (on the basis of the assay of pure, crystalline 'curling factor') that in the greatest dilution of culture filtrate showing waving of the hyphae there is a concentration of 0·16 μg./ml. of

'curling factor'. Considering the known inaccuracies of the assay method the actual yield follows the expected yield reasonably closely.

The effect of 'curling factor' on the germination of *Botrytis Allii* conidia can now be considered in greater detail. By observation of germination drops over a period of four days, it was found that in concentrations of 20 μg./ml. 'curling factor', a markedly stunted germ-tube is produced (see Fig. 2 A) and that growth ceases at that stage. In higher concentrations (e.g. 100 μg./ml.) germination may be a little delayed but almost complete germination does occur, growth ceasing once the short swollen germ-tube is produced.

Table 5. *Correlation between bio-assay and yield*

Batch no.	Activity by bio-assay	'Expected yield' (mg./l.)	Yield found (mg./l.)
42	$S_{16}C_{128}$	20	23
56	SoCo	—	—
111A	S_8C_{64}	10	—
117	$S_{16}C_{256}$	41	23
121	$S_{16}C_{128}$	20	9
149C	$S_{16}C_{256}$	41	37
47	S_8C_{512}	82	81
57	S_4C_{256}	41	24
111B	S_4C_{64}	10	8
118	$S_{16}C_{128}$	20	48
134A	$S_{32}C_{512}$	82	155
134B	$S_{16}C_{512}$	82	82
142A	$S_{128}C_{2048}$	327	151
149A	$S_{512}C_{2048}$	327	166
154A	$S_{128}C_{512}$	82	124
154C	$S_{32}C_{1024}$	164	65
149B	$S_{128}C_{4096}$	654	158
142B	$S_{32}C_{512}$	82	59
154B	$S_{32}C_{1024}$	164	51

When included in a Czapek-Dox agar medium the effect of 'curling factor' on germination and growth of *Botrytis Allii* is similar. At a concentration of 1 μg./ml. the colonies are much reduced in size (a diameter of 2 cm. after four days' growth at 25° C. as compared with 6 cm. in control), the rate of radial spread being much reduced. The advancing hyphae at the edge of such colonies are much waved, contorted and excessively branched. With concentrations of 10 μg./ml. and 25 μg./ml. no growth was visible to the naked eye, but examination of plate cultures microscopically showed that all spores had germinated, producing germ-tubes similar to those illustrated in Fig. 2 A–D; observations after seven days showed that no further development had taken place. If these germinated spores were then removed to normal Czapek-Dox agar, growth proceeded more or less normally.

Thus the physiological effects of 'curling factor' on *B. Allii* may be summarized as follows:

(*a*) In low concentrations (1 μg./ml. or less) growth is retarded, and the hyphae produced are excessively branched, waved and contorted.

(*b*) In higher concentrations (5–100 μg./ml.) germination of conidia is not prevented but growth ceases after a certain early stage of development of the germ-tube is reached, although death does not immediately occur.

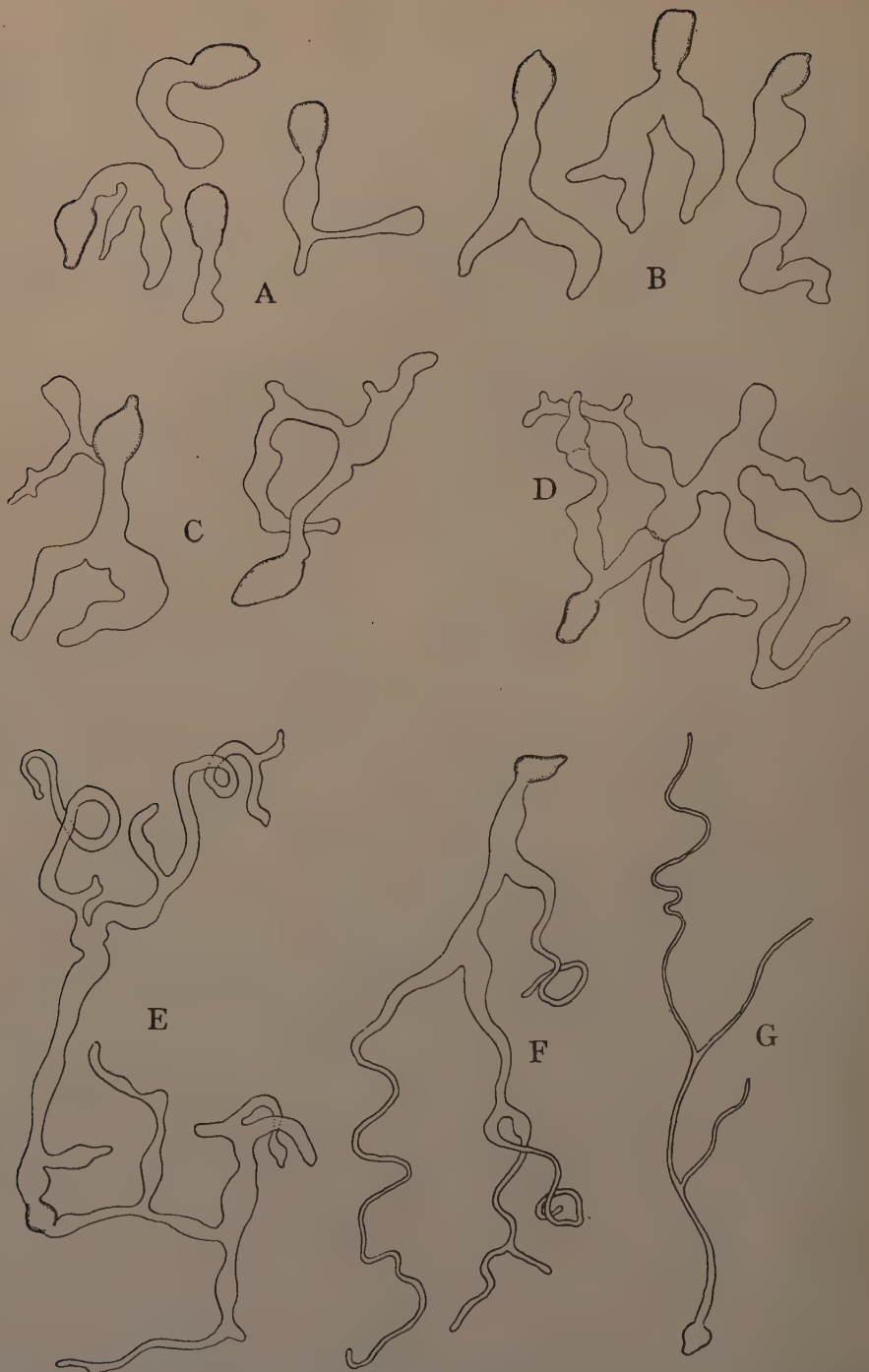


Fig. 2. Effect of various concentrations of pure crystalline 'curling factor' on germination of *Botrytis Allii*. Concentrations: A, 20 μg./ml.; B, 10 μg./ml.; C, 5 μg./ml.; D, 2·5 μg./ml.; E, 1·25 μg./ml.; F, 0·625 μg./ml.; G, 0·16 μg./ml. (A–F × 1·5: G × 1.)

Preliminary observations indicate that the effect of 'curling factor' on other fungi is qualitatively similar. No effects on growth of bacteria have been observed.

Discussion

The two most interesting physiological effects of 'curling factor' on *Botrytis Allii* are (*a*) inhibition of growth only at a stage subsequent to germination of the conidia and (*b*) the remarkable increase in branching and distortion of hyphae produced by low concentrations.

The use of the terms 'fungistatic' and 'fungicidal' has been discussed by McCallan & Wellman (1942), who point out that whereas ideally a fungistatic property may be defined as the ability to prevent growth of fungi without killing and a fungicidal property as the power to kill fungal spores or mycelium, in practice it is not possible to disentangle the two properties completely. A substance which is fungicidal at a given concentration is also fungistatic. However, one can in general terms distinguish between fungicidal substances, which actually kill fungi at concentrations close to those which merely produce a fungistatic or inhibitory effect, and fungistatic substances which prevent growth of fungi at concentrations well below that needed to cause a rapid killing effect. The distinction is probably concerned with the degree of reversibility of the inhibitory mechanism. 'Curling factor' is a typically fungistatic substance, using the term in the sense described above, but of an unusual type. It is fungistatic in so far as growth is markedly restricted at concentrations well below those needed to prevent germination or permanently to stop growth, but its effect is unusual in that the inhibition of growth is most marked at a stage subsequent to germination.

The effects of 'curling factor' on the growth form of *B. Allii* hyphae are unique. Many substances which have fungicidal or fungistatic activity may produce stunted and slightly distorted growth forms at concentrations just below that required to cause inhibition of growth. Boric acid is an example. 'Curling factor' is remarkable in that easily detectable effects on growth form are produced at concentrations far below that required to arrest growth. The distortion and excessive branching which it causes are of a much more pronounced type than any previously described as being caused by pure chemicals.

Similar morphological effects on fungi, caused by metabolic products of micro-organisms, have been noticed before. Porter (1924) has described the distortion of hyphae of species of *Helminthosporium* when growing near to colonies of a *Bacterium*. His description is very reminiscent of the effects of 'curling factor'—'when the hyphae of *Helminthosporium* are within 2 cm. of the bacterial colony, growth slackens and eventually ceases. Branches are given off from the hyphae in every direction and these become gnarled and twisted, and bubble-like enlargements of varying size appear in this portion of the mycelium'. Elliot (1917) has described distortions of species of *Alternaria* grown in the proximity of an unidentified *Bacterium*. Cook, Kreke, Giersch and Schroeder (1941) have prepared concentrates from yeast which, included in culture media, cause the formation of thick,

gnarled mycelia by fungi. Reinhardt (1892) and Buller (1933) illustrate a distortion and irregular branching of hyphae of *Sclerotinia sclerotiorum* when grown in the proximity of *Mucor*. It seems probable that substances causing abnormal morphological development of fungal hyphae are produced by a wide variety of micro-organisms.

Summary

Strains of *Penicillium Janczewskii* Zal. are shown to produce a substance, when grown on synthetic liquid culture media, which causes an unusual stunting and distortion of the germ-tubes and hyphae of *Botrytis Allii* and other fungi.

This substance, known as 'curling factor', is produced more abundantly in media containing nitrogen as nitrate than in media containing nitrogen as ammonium salts or peptone. The optimum concentration of glucose in the media is 7·5%, which is noticeably better than 5·0%. Increase in glucose concentration to 10·0% or higher gives no further advantage.

The 'curling factor' may be removed from active culture-filtrates by extraction with chloroform, ether or *n*-butyl alcohol or by treatment with activated charcoal followed by elution of the charcoal with ether or chloroform. By extraction with chloroform followed by successive crystallization from ethyl alcohol and dioxan/water mixtures, 'curling factor' has been obtained in pure form as colourless crystals. Yields of 150 mg./l. of culture filtrate can be obtained.

In concentrations of 25 μg./ml., 'curling factor' causes the production of short stunted germ-tubes by *Botrytis Allii*, whose development ceases at an early stage; lower concentrations (1·0 μg./ml.) cause excessive branching and distortion and lower concentrations still (0·2 μg./ml.) cause a waving of the hyphae. It is characteristic of the substance that relatively high concentrations (100 μg./ml.) do not prevent germination of *B. Allii* conidia, though far lower concentrations cause the profound physiological effects described.

We are much indebted to Miss Myrtle Bray for assistance with the bio-assays and to Mr George Elson for help in production and isolation of 'curling factor'.

REFERENCES

Abraham, E. P., Chain, E., Fletcher, C. M., Gardner, A. D., Heatley, N. G., Jennings, M. A. & Florey, H. W. (1941). Further observations on penicillin. *Lancet*, No. 241, 177–88.

Brian, P. W., Curtis, P. J., Hemming, H. G. & McGowan, J. C. (1946). The production of viridin by pigment-forming strains of *Trichoderma viride*. *Ann. appl. Biol.* xxxiii, 190–200.

Brian, P. W. & Hemming, H. G. (1945). Gliotoxin, a fungistatic metabolic product of *Trichoderma viride*. *Ann. appl. Biol.* xxxii, 214–20.

Brian, P. W., Hemming, H. G. & McGowan, J. C. (1945). Origin of a toxicity to mycorrhiza in Wareham Heath soil. *Nature, Lond.*, clv, 637.

Buller, A. H. R. (1933). *Researches on Fungi*, v.

Cook, E. S., Kreke, C. W., Giersch, M. C. & Schroeder, M. P. (1941). A growth-depressant substance from yeast. *Science*, xciii, 616–17.

Elliot, J. A. (1917). Taxonomic characters of the genera *Alternaria* and *Macrosporium*. *Amer. J. Bot.* iv, 439–76.

McCallan, S. E. A. & Wellman, R. H. (1942). Fungicidal versus fungistatic. *Cont. Boyce Thompson Inst.* xii, 451–63.

McGowan, J. C. (1946). A substance causing abnormal development of fungal hyphae produced by *Penicillium Janczewskii* Zal. 2. Preliminary note on the chemical and physical properties of 'curling factor'. *Trans. Brit. mycol. Soc.* xxix, 188.

Neilson-Jones, W. (1941). Biological aspects of soil fertility. *J. agric. Sci.* xxxi, 379–411.

Porter, C. L. (1924). Concerning the characters of certain fungi as exhibited by their growth in the presence of other fungi. *Amer. J. Bot.* xi, 168–88.

Reinhardt, M. O. (1892). Das Wachstum der Pilzhyphen. *Jb. wiss. Bot.* xxiii, 479–566.

Thom, C. (1930). *The Penicillia.*

(*Accepted for publication* 3 *June* 1946)

A SUBSTANCE CAUSING ABNORMAL DEVELOPMENT OF FUNGAL HYPHAE PRODUCED BY *PENICILLIUM JANCZEWSKII* ZAL.

II. PRELIMINARY NOTES ON THE CHEMICAL AND PHYSICAL PROPERTIES OF 'CURLING FACTOR'

By J. C. McGOWAN, *Hawthorndale Laboratories, Jealott's Hill Research Station*

In the preceding paper (Brian, Curtis & Hemming, 1946) an account is given of the isolation and production of a metabolic product of *Penicillium Janczewskii*, 'curling factor', which in low concentrations causes unusual stunting and distortion of germ-tubes and hyphae of *Botrytis Allii* and other fungi. The crude substance could not as a rule be satisfactorily purified by repeated crystallization from ethyl alcohol. However, after recrystallization from either aqueous 1 : 4-dioxane, or isobutyl alcohol, or aqueous tetrahydrofurfuryl alcohol and then from ethyl alcohol, colourless octahedral crystals were obtained. The melting point was 220° C. 'Curling factor' contained no nitrogen, no sulphur, and no halogens. Found: C, 59·3, 59·2, and 58·5%; H, 4·9, 5·0 and 4·9%; and mol. wt. (Rasts' method), 398, 387 and 378. $C_{20}H_{20}O_9$ requires: C, 59·4%; H, 5·0%; and mol. wt. 404.

'Curling factor' is optically active and the specific rotation $[\alpha]_D^{17}$ observed by sodium D light at 17° C., using a 2% solution in chloroform, had the very high value of +370°.

The pure 'curling factor' is very soluble in acetic acid, 1 : 4-dioxane, and benzene, is soluble in ether and ethyl alcohol and is only slightly soluble in ligroin. The octahedral crystals described above dissolve readily in cold chloroform but crystallization soon sets in and needle-shaped crystals separate. 'Curling factor' is only sparingly soluble in hot carbon tetrachloride and separates in needles when solutions in this solvent are cooled.

There are three methoxyl groups in each molecule of 'curling factor'. One methyl group attached to carbon is indicated and hydroxyl groups seem to be absent. Work on the chemical constitution is in progress.

The author wishes to thank Mr P. J. Curtis for preparation of the 'curling factor' with which to do this work.

REFERENCE

Brian, P. W., Curtis, P. J. & Hemming, H. G. (1946). A substance causing abnormal development of Fungal Hyphae produced by *Penicillium Janczewskii* Zal. I. Biological assay, production and isolation of 'curling factor'. *Trans. Brit. mycol. Soc.* xxix, 173–87.

(*Accepted for publication* 3 *June* 1946)

PROCEEDINGS

Meeting held in the Department of Biology, Chelsea Polytechnic, London, at 11.15 a.m., 23 February 1946. The President, Dr J. Ramsbottom, in the Chair.

G. C. Ainsworth. Notes on the taxonomy and nomenclature of British Smuts.
Miss K. Sampson. Notes on the biology of British Smuts.
O. De Rousset-Hall. Exhibit of agarics preserved by freeze-drying.
E. A. Ellis. *Symphyosira* Preuss. and two British Discomycetes with *Symphyosira*-like conidial organs.
P. W. Brian. Fungistatic substances produced by *Trichoderma viride*.
E. W. Mason. The name *Helminthosporium velutinum*.
S. J. Hughes. Some specimens called *Helminthosporium velutinum*.

Meeting held in the Board Room of the British Museum (Natural History), South Kensington, London, from 2 to 4 p.m., 12 April 1946. At 2.30 p.m., after a short business meeting, the President commented on a series of

Exhibits illustrating the development of British Mycology and the history of the Society

from the Museum collections and the Society's archives. The exhibits included the Berkeley and Browne correspondence, original drawings of fungi by noted botanical artists, a virtually complete series of mycological books published in this country during the past fifty years, photographs of past-Presidents, and a number of old foray photographs.

Meeting held in the Department of Botany, Glasgow University, at 10.30 a.m., 28 June 1946. The Chairman of the Plant Pathology Committee, Mr W. C. Moore, in the Chair.

A. E. W. Boyd, C. E. Foister and A. R. Wilson. Infestation of soils by *Fusarium caeruleum* in relation to the incidence of dry rot in potatoes.
Miss Elizabeth Gray. Problems in plant pathology in the North of Scotland.
C. H. Cadman. Raspberry pathology in Scotland.
H. F. Dovaston. The ascigerous stage of a species of *Epicoccum*.
Miss Mary Noble. A brief history of early mycological work in Scotland.
Malcolm Wilson. Alpine rusts in Scotland.
Mrs Elsie Conway. Exhibit of Japanese and British agars.